

BERT BJÖRKNER

*SENSITIZING CAPACITY OF ULTRAVIOLET CURABLE
ACRYLIC COMPOUNDS*

LUND 1984



From the Department of Occupational Dermatology,
Department of Dermatology,
University of Lund,
Lund, Sweden

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ACRYLIC COMPOUNDS

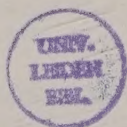
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To the memory of
Professor Bertil Magnusson

CONTENTS

CHEMICAL ABBREVIATIONS	7
AIMS OF THE STUDY	8
INTRODUCTION	9
Prepolymers	11
Multifunctional acrylates	15
Monofunctional acrylic monomers	19
Composition of UV-curable inks	21
The photoinitiator system	21
Effects of oxygen	24
Cross-linking agents	25
Inhibitors	25
UV-light sources	25
End-uses	26
MATERIAL AND METHODS	28
The Guinea Pig Maximization Test	28
High Performance Liquid Chromatography-analysis	33
Influence of the test vehicle	33
RESULTS	34
Prepolymers	34
Multifunctional acrylates	38
Monofunctional acrylates	40
HPLC-analyses	40
The influence of the vehicle	42
DISCUSSION	45
GENERAL SUMMARY	67
ACKNOWLEDGEMENTS	70
REFERENCES	71
PAPER I - IX	79



This thesis is based on the following papers, which will be referred to by their Roman numerals:

- I. Allergic contact dermatitis from acrylates in ultraviolet curing inks. Contact Dermatitis 1980: 6: 405-409
- II. Allergenicity of trimethylol propane triacrylate in ultraviolet curing inks in the guinea pig. Acta Dermato-Vener (Stockholm) 1980: 60: 6: 528-531
- III. Sensitization capacity of acrylated prepolymers in ultraviolet curing inks tested in the guinea pig. Acta Dermato-Vener (Stockholm) 1981: 61: 7-10
- IV. Sensitization capacity of polyester methacrylate in ultraviolet curing inks tested in the guinea pig. Acta Dermato-Vener (Stockholm) 1982: 62: 153-182
- V. The sensitizing potential of di(meth)acrylates based on bisphenol A or epoxy resin in the guinea pig. Contact Dermatitis. Accepted for publication
- VI. Contact allergy to 2-hydroxypropyl methacrylate (2-HPMA) in ultraviolet curable ink. Acta Dermato-Vener (Stockholm) Accepted for publication
- VII. Sensitizing potential of urethane (meth)acrylates in the guinea pig. Contact Dermatitis. Accepted for publication
- VIII. The sensitizing capacity of multifunctional acrylates in the guinea pig. Contact Dermatitis. Submitted for publication
- IX. Influence of the vehicle on elicitation of contact allergic reactions to acrylic compounds in the guinea pig. Contact Dermatitis. Submitted for publication

CHEMICAL ABBREVIATIONS

Chemical abbreviations of acrylic compounds used in this investigation.

Abbreviation	Chemical name
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Prepolymers

Epoxy acrylate	2,2-bis(4-(2-hydroxy-3-acryloxypropoxy)phenyl)propane
BIS-PMA	2,2-bis(4-(3-methacryloxypropoxy)phenyl)propane
BIS-EMA	2,2-bis(4-(2-methacryloxyethoxy)phenyl)propane
BIS-GMA	2,2-bis(4-(2-hydroxy-3-methacryloxypropoxy)phenyl)propane
BIS-MA	2,2-bis(4-(methacryloxy)phenyl)propane
UEDMA	Urethane methacrylate

Multifunctional acrylates

BUDA	1,4-Butanediol diacrylate
HDDA	1,6-Hexanediol diacrylate
DEGDA	Diethylene glycol diacrylate
TREGDA	Triethylene glycol diacrylate
TEGDA	Tetraethylene glycol diacrylate
TPGDA	Tripropylene glycol diacrylate
NPGDA	Neopentyl glycol diacrylate
BUDMA	1,4-Butanediol dimethacrylate
HDDMA	1,6-Hexanediol dimethacrylate
EGDMA	Ethylene glycol dimethacrylate
TREGDMA	Triethylene glycol dimethacrylate
TEGDMA	Tetraethylene glycol dimethacrylate
NPGDMA	Neopentyl glycol dimethacrylate
PETA-3	Pentaerythritol triacrylate
OTA 480	Oligo triacrylate
TMPTA	Trimethylolpropane triacrylate
TMPTMA	Trimethylolpropane trimethacrylate

PETA-4	Pentaerythritol tetraacrylate
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Monofunctional acrylates

2-HEA	2-Hydroxyethyl acrylate
2-HPA	2-Hydroxypropyl acrylate
2-EHA	2-Ethylhexyl acrylate
2-HEMA	2-Hydroxyethyl methacrylate
2-HPMA	2-Hydroxypropyl methacrylate

AIMS OF THE STUDY

The purposes of the present work were as follow:

- To investigate the cause of dermatitis among workers exposed to UV-curable inks as various printing plants.
- To investigate the sensitizing capacity of various acrylic compounds commonly used in UV-curable inks or coatings by means of "The Guinea Pig Maximization Test" (GPM-test).
- To check the purity of the acrylates tested using High Performance Liquid Chromatography (HPLC)-analyses.
- To isolate and collect the main fractions of the di(meth)acrylates based on bisphenol A or epoxy resin on HPLC for further GPM-tests.
- To study the possible sensitizing potential of the impurities.
- To elucidate the influence, the various vehicles might have on the challenge response.
- To compare the challenge response at various patch test locations.
- To establish, from the results in this study, the preventive measures necessary to eliminate skin hazards from UV-curable acrylic compounds.

INTRODUCTION

Photopolymerization might be defined as the process whereby light is used to induce an increase in molecular weight. This process involves for example the chain reaction polymerization of a vinyl monomer ($\text{CH}_2=\text{CH}-$). Another type of photopolymerization is concerned with the cross-linking of preexisting high polymers. Following the common practice, the former process is referred to as photopolymerization and the latter as a photocrosslinking. The distinction between these two processes is not always clear (68). The history of photopolymerization is old, beginning with the use of sunlight to dry Egyptian mummy wrappings. Linen cloths were dipped into a solution of "oil of lavender" containing Syrian asphalt. Syrian asphalt, also known as "bitumen of Judea" is a high-molecular-weight bituminous material which, the Bible says, was used in the Ark of Noah and in the rush basket of the infant Moses. On exposure to sunlight the substance undergoes cross-linking (68).

The ability of light to initiate the polymerization of vinyl monomers has been known for over a century. Early works, describes vinyl monomers exposed to sunlight, thereby obtaining high polymers. Not until 1910, ultraviolet light was used for polymerization, when ethylene, and thereby the first polyethylene, was obtained in solid form (68). During the past decade there has been a growing interest in photochemical applications to polymers. The radiation curing is a technology which uses electromagnetic (mainly ultraviolet light) or ionizing radiation (accelerated electrons) to initiate a chain mechanism by which mixtures of polyfunctional compounds are transformed into a cross-linked polymer network.

The first patent on ultraviolet (UV) light-cured printing inks was granted in 1946 (86). About the same period General Electric Company was experimenting with ionizing radiation to cure solventless coatings.

The development of more powerful and efficient UV-light sources and electron accelerators, the progress made in photo-chemistry technology and the increasing availability of a wide range of acrylic acid derivatives have all contributed to, the

fact that a large number of patents have been granted in the UV-curable polymer field. However, a few patents are dated many years back e.g., those for most UV-curable polymers and coating compositions have been issued since 1969 (78).

After the oil crisis 10 years ago, the requirements for more efficient use of energy were made. About the same time there was a growing concern about air pollution, occupational safety and health hazards, factors that have forced the industry to develop new technologies. UV-curing requires up to 90% less energy than thermal curing (78, 79).

In the conventional thermal system, the decided polymer is prepared in a separate stage from monomers and/or intermediates in a solvent. The polymer solution is applied on the substrate and heated to decipate the solvent, leaving the solvent coating on the substrate. For UV-curing, a different sequence of steps and a different source of energy are employed to arrive at the same end result. Unlike thermal curing, the UV- or electron beam curing process, does not require heat and no solvent is emitted. Since no solvent has to be evaporated from such systems prior to cure, little if any pollution might be expected from such resins.

Thus, two fundamental differences distinguish radiation-cured systems from conventional systems based on polymers in solutions or aqueous dispersions.

- These are:
- i) The absence of volatile solvents.
 - ii) The mechanism of polymerization of the components of the formulation under the influence of irradiation.

The chemical process with the formation of chemical bonds is substituted for a physical process with evaporation of solvents.

The simplest UV-curable formulation can consist of only three components, but in practice a typical industrial formulation contains a much higher number of ingredients. The three essential components are: a UV-reactive prepolymer, that provides the bulk of the desired properties, a diluent system composed of multifunctional acrylate esters (and, at times, monofunctional acrylic esters) and the photoinitiator system (76, 78, 79).

The compositions of many UV-curable resins are quite similar to those of systems prepared to electron beam curing (EBC) (77). UV-irradiation is better suited to non-pigmented or slightly pigmented systems having relatively thin films to allow full penetration of the irradiation. For pigmented coatings, polymerization is best achieved by electron beam curing. UV and EBC are supplements to each other in many respects. The UV method may be used more advantageously, for example, in the cure of shaped articles since UV lamps can easily be arranged to surround any given configuration. It may also be used for substrates such as paper or certain plastics, which are degraded or discoloured by electron beam radiation (77).

The prepolymers in UV-curable resin formulations can be divided into two broad classes: unsaturated polyester resins and polymers containing pendant unsaturation. In an unsaturated polyester resin, the monomer or "solvent" is primarily styrene. Peroxide-cured unsaturated polyesters have been used commercially for many years, but UV-curable unsaturated polyesters are quite similar in composition to resins used commercially for peroxide cures. The UV-curable polyester system is used in the furniture industry (77).

THE PREPOLYMERS

This study will focus on the second major class of UV-curable prepolymers. They may be based on any of the conventional thermoplastic resins into which the very reactive acrylate or methacrylate groups have been introduced. The reactive (meth)acrylate groups are attached to the backbone of the resins through a functional group such as hydroxyl, carboxyl, amine, isocyanate, anhydride or epoxy. The most commonly used prepolymers in the UV-curing field are: acrylated epoxies, acrylated polyurethanes, acrylated polyesters, acrylated polyethers, acrylated acrylics and acrylated alkyds (31, 39, 76, 78, 79, 87, 88).

Epoxy acrylates. The β -hydroxyester acrylates are also called epoxy acrylates, because they are usually obtained by reacting epoxy resins or glycidyl derivatives with acrylic acid (Fig. 1). Both aromatic or aliphatic epoxy acrylates are available as well as acrylated epoxidized oils. The properties of resins and acrylates combined, have a major influence on their utility in radiation curable systems. The epoxy resin part provides the toughness, adhesion and chemical resistance, and a terminal acrylic functionality imparts high free radical reactivity. Epoxy acrylates have their most useful applications where moderate adhesion to metal surface is required and on wood, particle board and paper coatings and in printing inks where,

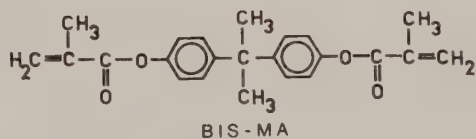
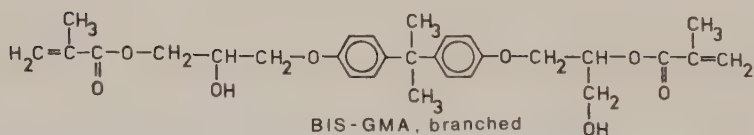
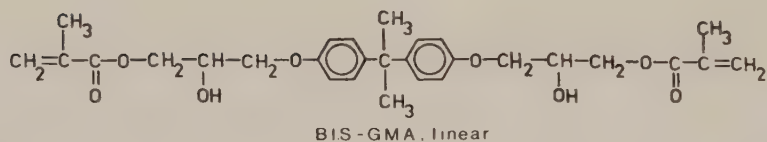
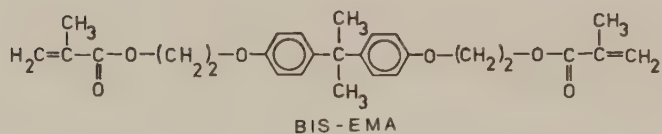
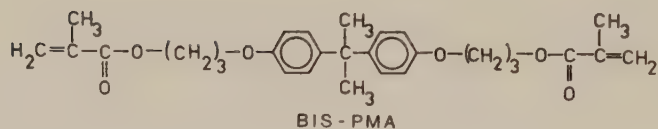
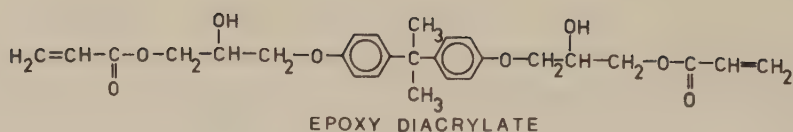


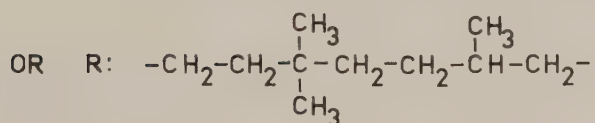
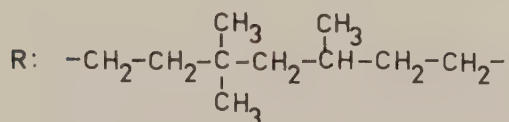
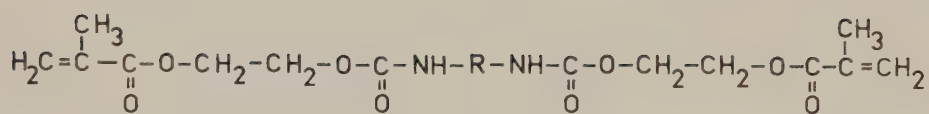
Fig. 1. Chemical structure of di(meth)acrylates based on bisphenol A and epoxy resin.

a highly reactive system is desired (76, 84).

BIS-GMA (2,2-bis[4-(2-hydroxy-3-methacryloxypropoxy)phenyl]propane) is the addition reaction product of bisphenol A and glycidyl methacrylate or an epoxy resin and methacrylic acid (13, 15, 75, 86). BIS-GMA can thus be classified as a dimethacrylated epoxy. BIS-GMA is the most commonly used prepolymer in dental composite restorative materials (58). Several variants have also appeared as substitutes for BIS-GMA or in addition to BIS-GMA in dental resins. Such dimethacrylates based on bisphenol A with various chain lengths are BIS-MA (2,2-bis[4(methacryloxy)phenyl]propane), BIS-EMA (2,2-bis[4-(2-methacryloxyethoxy)phenyl]propane) and BIS-PMA (2,2-bis[4-(3-methacryloxypropoxy)phenyl]propane). In addition to serving as the principal monomer in dental composites and sealants, BIS-GMA resins and the other similar derivatives are finding extensive use in industrial applications (14, 16, 41, 54, 55, 96, 97). Acrylates based on bisphenol A or epoxy resin can be polymerized not only by exposure to electron beams, ultraviolet light or even visible light (21), but also chemically activated by the use of various peroxides. The structural formulas of di(meth)acrylates based on bisphenol A or epoxy resin are shown in Fig. 1.

Urethane acrylates. Of the many various types of acrylated urethanes on the market, some are based on aromatic isocyanates while other are of the aliphatic types. They can vary in functionality with acrylated or methacrylated groups and with various molecular weights. The acrylated urethanes are not only used as prepolymers in UV-curable coatings, as for instance vinyl flooring, but also as resins for dental applications. The acrylated urethanes used in dentistry are mostly of the methacrylated types and they play the same role as the more common BIS-GMA in composite and sealant formulations (28, 75). The proposed formula of an urethane dimethacrylate (UEDMA) and its isomers is shown in Fig. 2.

Polyester acrylates. Properly UV-cured unsaturated polyesters have been found to give properties equivalent to peroxide-cured systems. The UV-method therefore has found its first application in the cure of these materials. Polyester acrylates can be produced in a wide range of viscosities and reactivities to adapt to end uses in printing inks for wood and paper coatings (100).



AND MANY OTHER POSSIBILITIES

Fig. 2. The proposed formulae of urethane methacrylates (UEDMA) and its isomers.

Polyether acrylates. The main advantage of polyether acrylates is the possibility to prepare reactive oligomers with low viscosity (100).

Acrylated acrylics. The acrylated acrylics finally represent prepolymers with an acrylic backbone on which unsaturated acrylic groups are grafted. These resins present an outstanding resistance for outdoor exposure (100).

Acrylated alkyds. These are less common in the UV-curable resin formulations.

Thus, the polyether acrylates, acrylated acrylics and acrylated alkyds are the prepolymers in general less frequently used.

Curing speeds of the prepolymers are of the same general order, since all are of the acrylated type. Coatings derived from prepolymers, having methacrylic unsaturation, are also contenders as UV-curable intermediates. For total cure, such prepolymers require higher energy than materials with acrylic functionality. However, the problem is minor as rapid curing can be achieved with higher irradiation or a short thermal post-cure (76).

MULTIFUNCTIONAL ACRYLATES

Most of the acrylated prepolymers tend to be quite viscous. Additional, unsaturated sites of the prepolymer itself, may not be high enough in number to produce adequate cross-linked density for most applications. Use of the multifunctional acrylates alleviates both these short-comings. These low viscosity monomers act as cross-linking agents and reactive diluents and becoming a part of the final coating on UV-exposure.

The multifunctional acrylates are for example di(meth)acrylate esters of dialcohols or tri- and tetraacrylate esters of polyalcohols. Apart from being used in formulations for UV-curable inks or electron beam irradiation curable coatings, the multifunctional acrylates are important acrylic compounds in photopolymers, flexographic printing plates (48, 51, 53, 60). and photoresists (an etch resist for printed circuit boards) (23). In acrylic glues, adhesives and anaerobic sealants as well as in artificial nail preparations, the multifunctional acrylate esters are useful (27, 48). Some of the more commonly used are ethylene glycol dimethacrylate (EGDMA), diethylene glycol dimethacrylate (DEGDMA) and trimethylolpropane trimethacrylate (TMPTMA) (27).

Most of the dental composite resin materials and denture based polymers are "diluted" with less viscous "difunctional" acrylates. These are the methacrylic monomers, of which EGDMA, DEGDMA, triethylene glycol dimethacrylate (TREGDMA) and 1,4-butanediol dimethacrylate (BUDMA) are used most extensively (1, 32, 73, 74, 75). (Fig. 3 and 4.)

The most commonly used multifunctional acrylate in an UV-curable ink or coating formulation, is an acrylic acid ester of either pentaerythritol (PETA), trimethylolpropane (TMPTA) or hexanediol (HDDA) (76). The commercially available PETA is a mixture of pentaerythritol tri- and tetraacrylates. (Fig. 5). PETA has therefore the highest degree of acrylation (an average of 3.3 acrylate groups per mole) and provides the highest degree of cross-linked density. PETA improves cure speed, hardness and gloss, along with providing viscosity reduction. PETA has found to be useful in a variety of UV-cure applications and is especially favoured for UV-cured letterpress and flexographic inks (78).

Trimethylolpropane triacrylate (TMPTA) (Fig. 6), incorporates most of the PETA's desirable features in addition to having a lower viscosity and providing a higher degree of flexibility than does PETA. This combination of high reactivity and low viscosity gives the formulator considerable freedom in producing a coating with high performance and ease of application (78).

Hexanediol diacrylate (HDDA) (Fig. 4) has the lowest viscosity of the three multifunctional acrylates and can bring about a significant viscosity reduction in UV-curable formulations. HDDA is comparable to some acrylic monomers in its ability to reduce viscosity (31). HDDA might be expected to find applications in such areas as UV-curable gravure inks, sprayable coatings and top coats for flexible vinylstock. Inclusion of HDDA markedly improves elasticity and adhesion (78).

Thus, the addition of small amounts of PETA or TMPTA, will greatly increase the hardness, but adhesion to metallic surfaces drops rapidly as the amount of trifunctional acrylates are increased. The trifunctional acrylates, while unacceptable on metallic substrates, may be quite acceptable for increasing the hardness of coatings on such rigid substrates as wood (31). It is apparent that the choice of the multifunctional acrylate is dependent upon the ultimate use of the coating and ink and the method of application. The 3 multifunctional acrylates described here, cover a broad range in terms of performance and viscosity and in addition, blends of

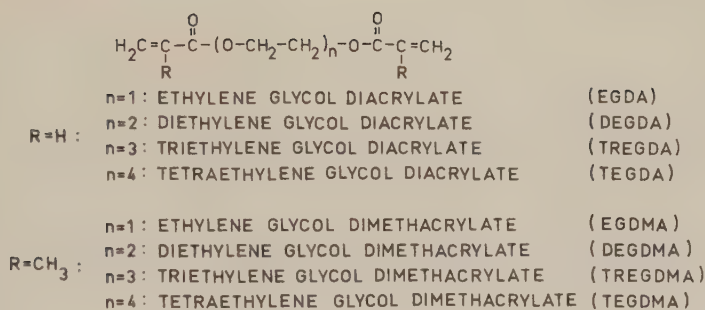


Fig. 3. Chemical structure of n-ethylene glycol di(meth)acrylates.

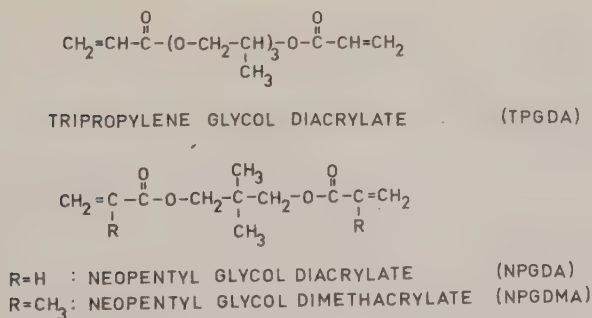
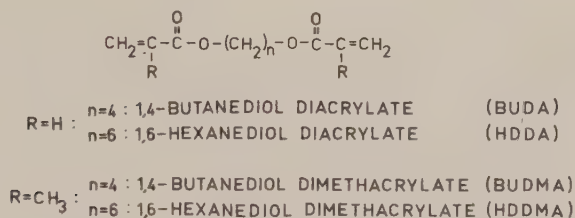
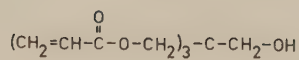
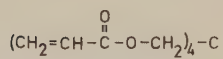


Fig. 4. Structural formulae of di(meth)acrylates based on butanediol, hexanediol, tripropylene glycol and neopentyl glycol.

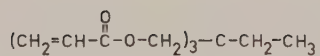


PENTAERYTHRITOL TRIACRYLATE (PETA-3)



PENTAERYTHRITOL TETRAACRYLATE (PETA-4)

Fig. 5. Structural formulae of pentaerythritol tri- and tetraacrylate.



TRIMETHYLOLPROPANE TRIACRYLATE (TMPTA)

Fig. 6. Structural formulae of trimethylolpropane triacrylate.

these monomers will be used to provide the specific features desired in any application.

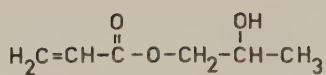
MONOFUNCTIONAL ACRYLIC MONOMERS

Reactive acrylic monomers are included in the coating to facilitate application and for their contributions of the properties of cured coating. The reactive monomers reported in literature are all members of the vinyl monomer family (80). The ratio of monofunctional monomers to the multifunctional acrylates will influence on the curing speed. Monomer structure has a profound influence on the coating properties (80). Coating properties will be improved by combining multifunctional acrylates with reactive acrylic monofunctional monomers as they contribute to lower viscosity, toughness and adhesion to the coating (76). They also promote the disappearance of double bonds due to mutual reaction of backbones and cross-linkers (53). However, too high an amount of monofunctional acrylates can quench a photoinitiator and reduce its efficiency, because monomers polymerize at a much slower rate than di- and trifunctional unsaturated oligomers (56).

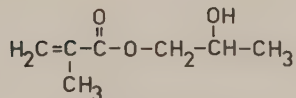
The most common monofunctional acrylates in UV-curable resin formulations are 2-ethylhexyl acrylate (2-EHA), 2-hydroxyethyl acrylate (2-HEA), 2-hydroxypropyl acrylate (2-HPA), 2-hydroxyethyl methacrylate (2-HEMA) and 2-hydroxypropyl methacrylate (2-HPMA) (31, 53, 78, 79). (Fig. 7).

UV-cured films show a greater tendency to yellow than those cured by EBC. Yellowing is reduced by increasing the proportion of pure acrylic diluent such as 2-EHA, 2-HPA or TMPTA (31). 2-HPA and 2-HPMA are hydrophilic in nature, therefore they are suitable for interior applications. Consequently, because of its hydrophilic properties, 2-HPMA is useful in formulating isotonic adhesive resins, e.g. prosthetic appliance formulations for dentistry or orthopedic surgery (15).

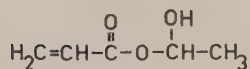
Other UV-curable applications for vinyl monomers, preferably hydroxyalkyl acrylates and methacrylates are impregnated fabric article, e.g. bandages (20).



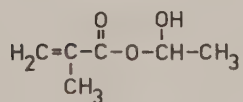
2-HYDROXYPROPYL ACRYLATE



2-HYDROXYPROPYL METHACRYLATE



2-HYDROXYETHYL ACRYLATE



2-HYDROXYETHYL METHACRYLATE

Fig. 7. Structural formulae of 2-hydroxypropyl acrylate (2-HPA), 2-hydroxypropyl methacrylate (2-HPMA), 2-hydroxyethyl acrylate (2-HEA) and 2-hydroxyethyl methacrylate (2-HEMA).

Apart from being used in UV-curable resin formulations for technical purposes, the reactive monofunctional monomers, most commonly the methacrylated ones, are used in UV-sensitive compositions for fissure sealants in dentistry (73).

COMPOSITION OF UV-CURABLE INKS

In a UV-curable ink, the various acrylic resins described previously are important components, but when formulating an ink for UV-cure, the prepolymer and the multifunctional and/or monofunctional acrylates are for practical purposes not sufficient. Usually the formulations contain five or more ingredients, as shown below:

- Prepolymers
- Multifunctional acrylates
- Monofunctional acrylates
- Photoinitiators
- Pigments
- Fillers
- Non reactive inert polymers
- Polymeric waxes
- Cross-linking agents

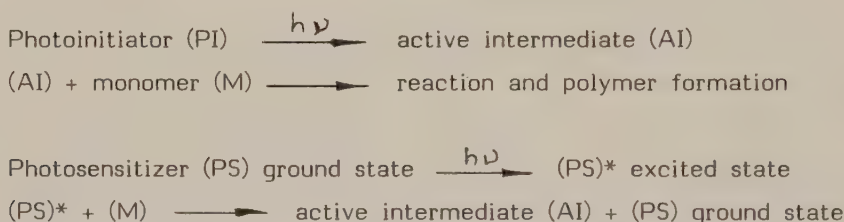
A typical UV-curable ink consists of 50 - 70% reactive vehicle (prepolymer, multifunctional acrylate, monofunctional acrylate), 5 - 20% photoinitiator, 10 - 30% pigments and the rest of other additives (79).

THE PHOTOINITIATOR SYSTEM

The two types of radiation used for polymerization are ultraviolet light (wavelength 150 - 450 nm) and accelerated electron beams (150-350 KeV). For UV-cured formulations, the photoinitiator is an essential component, while inks or coatings for EBC, the initiator system are unnecessary. The most photoinitiators absorb UV-light between 310 - 430 nm and thus the area of interest for UV-curing (79).

The majority of UV polymerizations of commercial interest are free radical chain reactions (67). Formation of free radicals must be produced by the addition of photochemically reactive substances known as photoinitiators and photosensitizers (not a photosensitizer from a dermatological point of view) (39, 40, 42).

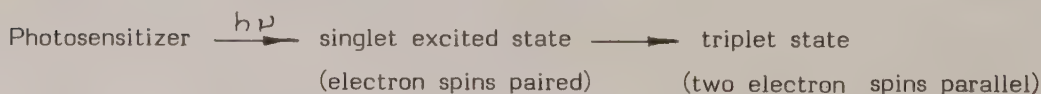
Thus, when a polymerizable monomer is exposed to UV light in the presence of a photoinitiator system, polymerization may be initiated by radicals generated by dissociation of the excited photoinitiator, or the excited photosensitizer may transfer its excitation energy to the monomer which, in turn, undergoes excitation and radically initiated polymerization (57). Radicals are molecules or atoms with an unshared electron. The radical reacts with the monomer by addition. A monomer-radical is formed, bearing an unshared electron. Through propagation, large polymer-radicals are produced. The growth of the polymers is terminated for instance when two polymer-radicals combine.



The basic difference between the two mechanisms is that, in one case the photoinitiator is physically changed or destroyed, while in the other case, the photosensitizer is not consumed but only acts as an energy transfer agent (57).

Free radical generation by UV-absorbing photoinitiators or photosensitizers is believed to occur by two basic mechanisms:

1. Homolytic fragmentation of the photoexcited molecule, giving a pair (intramolecular process).
2. Intermolecular hydrogen abstraction by a triplet excited photosensitizer from a hydrogen donor.



A great number of compounds have been patented as photoinitiators and photosensitizers but those which have obtained commercial significance belong mainly to the following classes (5, 42, 57, 70, 72).

- A.
 - 1) Benzoin ethers
 - 2) Benzil ketals
 - 3) Acetophenone derivatives
 - 4) Alpha-Acryloxime esters
- B. Ketone - (Ketone-Amine combinations)
 - ex. Benzophenone
 - Michler's ketone
 - Thioxanthone
 - Halogen-substituted alkyl-arylketone
 - Benzils
 - Quinone derivatives

The first type of initiators (A) is mainly used in clear lacquers (34). The second class of photoinitiators (B), which includes various aromatic ketones and quinones is usually combined with tertiary aliphatic amines as hydrogen donors. They are often used in pigmented coatings (5, 34). Combination of benzophenone and Michler's ketone (4,4-dimethylaminobenzophenone), represents an effective photoinitiator system for UV-curing of printing inks (70).

Benzil and quinones are photoinitiators suitable for UV-curable orthopedic devices and dental materials (70).

Through the introduction of a photosensitizer, α -diketones, which are capable of being excited by radiation in the visible region of the spectrum, that is, by light having a wavelength greater than 400 nm, an acrylic resin can be formulated. The composition can be formed into a paint-film or used as composite resin materials which then can be cured by natural light or sunlight (21).

The choice of a proper photoinitiator system for UV-curing of pigmented system has some difficulties. The absorption of the pigments in the near UV-range, between 300-400 nm, may effect the curing rate. The white pigmented inks have offered the most serious problems in UV-curing due to the high levels of pigmented needed in order to obtain good hiding power.

The main class of photoinitiators used in this area is thioxanthone and its derivatives together with tertiary amines (5, 30, 34).

Benzophenone and aliphatic amines both develop a characteristic odour. This is avoided by the use of a combination of acrylated benzophenone and acrylated amines.

Titanium dioxide pigmented systems are very opaque to UV up to approximately 370 nm. A photoinitiator absorbing in the upper UV-region is therefore necessary (33). Thus, the selection of a proper photoinitiator for UV-curing acquires careful consideration.

EFFECTS OF OXYGEN

One factor that must be considered is the inhibition and chain termination effects of oxygen in free radical chain reactions. Polymers which have a hydrogen atom on the carbon bearing the side chain, tend to cross-link, while those having substituents, like methyl or chlorine tend to degrade under the influence of oxygen (59). Because of this, UV-curable coatings must be cured in an oxygen-free atmosphere. The oxygen-free atmosphere may be attained in an inert gas chamber, or by covering the reactive ingredients with a UV-transparent- O_2 -impermeable cover, such as a polymer foil or a wax ingredient that migrates to the surface of the wet coating. The inert blanketing technique has been limited to some extent at the cost of nitrogen, which is used in large quantity. Another possibility for solving the air inhibition problem is to add tertiary aliphatic amines as co-initiators, which will diminish the effect the atmosphere may have on UV-curing (5). Alternative methods used to overcome air inhibition include the addition of phosphorus compounds, isocyanates or drying oils (77).

The problems of the air inhibition on surface cure are solved for most of the UV-curable formulations used today. Therefore, it is unlikely, that any acrylic monomers are left uncured on the surface. However, further investigations are needed.

CROSS-LINKING AGENTS

Cross-linking agents such as melamine-formaldehyde resins find some utility in operations where UV-curing is followed by short thermal bake, such as can coating operations. In this case, advantage can be taken of additional cross-linking between the melamine and their reactive sites on the polymers to achieve thorough curing of highly pigmented coatings (79).

INHIBITORS

Inhibitors are important ingredients in any formulation involving free radical chemistry. Inhibitors act to retard or prevent polymerization until cure is desired, such as occurs at the time of exposure to UV-light. Since the same inhibitors neutralize the free radicals generated by photosensitizers, a compromise must be made between package stability, on the one hand, and the retardation of the cure on the other.

All unsaturated resins and monomer diluents contain a small amount of inhibitors, usually 15-400 ppm (85). The amounts of inhibitors required are very small and too much may retard cure unnecessarily or otherwise destroy or reduce performance properties. Among the most useful inhibitors are the phenolic types. In this respect, hydroquinone, or one of its several derivatives, is especially useful, because it is highly effective over the range of temperatures used for formulating and storages of the resins, and because it exhibits a minimum retardation of UV-cure rate. Some of the derivatives of hydroquinone which may prove useful include hydroquinone monomethylether, toluhydroquinone, mono-t-butylhydroquinone and 2,5-di-t-butylhydroquinone (85). Another inhibitor added to acrylic monomers is p-methoxyphenol (93). Benzoquinone and related substances have rather significant retardation effect on UV-cure rate and should be used only in small amounts, if at all.

UV LIGHT SOURCES

The UV photons needed for polymerization are generated by lamps. Standard UV-lamps, suitable for UV-curing, are commercially available from lamp manufacturers. Both medium and high pressure mercury arcs, as well as special low pressure mercury lamps coated with phosphor (black light), are employed

commercially as UV-sources. Most frequently used type of lamps are medium pressure mercury arcs with the power input of 80-200 Watt/cm. They are available in length from a few cm, to over 2 m. They produce about 50 times the UV-intensity of the black light lamps. Mercury arcs are therefore the sources of the choice where maximum cure speeds are desired. However, these lamps emit a high proportion of infrared and visible light and generate a considerable amount of heat. They have to be cooled with air, which at the same time removes ozone from the working area. In many units, the reflector housing is water-cooled. Medium pressure lamps emit over the whole ultraviolet with a fixed spectral distribution. The spectral power distribution of black lamps on the other hand can be varied in the near ultraviolet and these offer the possibility of matching maximum lamp output with the action spectrum of the photoinitiator (77). Medium pressure mercury arcs have a slow burn-in time and need a considerable time to reach optimum output. Usually two types of reflectors are used, one semielliptical reflector, which focus all light into one line, and one parabolic reflector, which emit a parallel beam of light (100). To concentrate irradiation into peaks, pulsed medium pressure mercury lamps are used. Other mercury lamps are electrodeless arcs which will start up instantaneously with microwaves. The total length is limited to 25 cm but they have a longer lifetime than the medium pressure mercury arcs. Capillary high pressure mercury lamps have a very high output power, but are very expensive and short lived. They have a quick start but are only available in short length up to 20 cm. Still higher UV-intensities than with the present mercury arcs, are available with pulsed Xenon flash lamps or Plasma arcs. However, for economic as well as safety reasons, these UV-sources are not recommended for use in commercial curing systems. Mercury lamps, which emit only at 254 nm alone, are not useful for paint curing since wavelengths below 300 nm only penetrates the very first thin layer of the resin (77). Finally, the so called "doped" lamps, have been developed. Metal halides are introduced in the mercury vapour and capture photons which are reemitted at different wavelengths. The emission spectrum may be adjusted to match the absorption spectrum of the photoinitiator or the transmission spectrum of a pigmented coating.

END-USES

In the last decade the application of radiation curing technology has been extremely successful in many areas. In the beginning, commercial UV-curing

applications were used primarily in the furniture area. The last years' research effort in the coating industry by developing improved polymers and better lamps and photoinitiators, has focused the technique to other areas, as the can, electrical, plastic, paper and printing industry. The change from conventional inks to inks "dried" by UV-light has had a significant impact on the printing industry. Introduction of UV-curable inks has been made in many printing processes like lithographic, offset, letterpress, flexographic and silk screen printing applications. The main advantages in the printing ink field are a much lower energy consumption and the elimination of solvent emission (39).

In the can coating industry, UV-printing inks are used on beverages and beer cans as well as on crown caps and aerosol cans for technical, but also for important economic advantages.

Overprint varnishes have taken over substantial parts of the conventional wet solvent varnishes and film laminates.

The use of UV-curable coatings as wood finishing is a favourable technology. Wood-like materials include fibre boards and plastics, such as polystyrene, PVC and polyesters. Other areas with increased importance for UV-cure are mat varnish, parquet varnish and sealers which may contain up to 65% fillers, transparent to UV-light.

PVC foils can be printed and textured to give a surface which closely matches the appearance and feel of real wood. They can be glued and are suitable for the production of panels. These panels are used in the manufacture of TV and stereo cabinets. Other areas are laminating adhesives to paper, PVC and carton.

UV-curable acrylic compounds are also used in other areas than in inks, varnish and coatings. They are also useful in photoprepolymer printing plates and photo-elastomers for flexographic printing plates (53, 60). The main feature which distinguishes flexographic from other relief plates is their flexibility. Multifunctional acrylic compounds are used in photoresists (an etch resist for printed circuit boards) (23). UV polymerization are also used on dental composite resin materials and surgical casts (20, 69).

UV curing is probably still in its infancy, with major improvements yet to come. Improved and properly designed and formulated systems, air pollution regulations and fuel shortages are factors that will put UV-curing technology in the forefront. Predictions are, that as much as 20% of all industrial finishes may be cured by some form of radiation in a near future (77). Therefore, increased health hazards among the consumers caused by the various acrylic compounds, are also to be expected.

MATERIAL AND METHODS

THE GUINEA PIG MAXIMIZATION TEST (GPM-TEST)

The GPM-test was performed in accordance with the original description (44,45).

Animals. Female albino guinea pigs of the Hartley-Dunkin strain, weighing 300 - 400 g, were used. Approximately 30 guinea pigs were divided into one experimental group and one control group for the substances tested. The exact number of guinea pigs used in the various experiments are shown in the papers II - IX.

Chemicals. Tests were performed with commonly used acrylated prepolymers like polyester acrylate (IV), urethane (meth)acrylates (VII) and di(meth)acrylates based on bisphenol A or epoxy resin (III, V). The multifunctional and monofunctional acrylates tested are discussed in paper VIII and VI resp. Induction and challenge were performed with the commercial and/or pure acrylic products. Other relevant chemicals were also used for challenge. Detailed description of the various chemicals tested and their purity are described in detail in the various papers (II - IX). The degree of purity used for the acrylic substances for induction and challenge is shown in Table 1 and 2.

Topical irritancy. The topical irritancy of the chemicals tested was studied by a 24 h. closed patch test in animals not used in the test procedure. Challenge patch test concentrations were used, which did not give any reactions.

Intradermal reaction and systemic toxicity. Preliminary investigations were performed in 6 animals with 3 different concentrations to establish optimal sensitization of the test substance for intradermal induction without causing systemic toxicity.

Table 1. THE PREPOLYMERS USED FOR INDUCTION AND CHALLENGE AND THEIR DEGREES OF PURITY.

Chemical	Trade name	Purity
<u>Prepolymers</u>		
Polyester acrylate	Ebecryl 810	c
Epoxy acrylate	G-MAN	c
Bisphenol A dimethacrylate	BIS-EMA (G-MAN)	c
Epoxy acrylate	Epoxy acrylate DOW	c, m
Epoxy acrylate	Epikote acrylate DRH-370	c, m
BIS-PMA	BIS-PMA	98%
BIS-EMA	DIACRYL 101	c, m
BIS-GMA	BIS-GMA	c, m
BIS-MA	BIS-MA	c, m
Urethane acrylate	Ebecryl 220	c
Urethane acrylate	Ebecryl 270	c
Urethane methacrylate	UEDMA /	98%

c = commercial grade

m = main peak

Table 2. THE MULTIFUNCTIONAL AND MONOFUNCTIONAL ACRYLATES
USED FOR INDUCTION AND CHALLENGE AND THEIR DEGREES OF
PURITY.

Chemical	Trade name	Purity %
<u>Multifunctional</u>		
<u>-diacrylate</u>		
1,4-butanediol	BUDA	98
1,6-hexanediol	HDDA	90
diethylene glycol	DEGDA	95
triethylene glycol	TREGDA	95
tetraethylene glycol	TEGDA	98
tripropylene glycol	TPGDA	70
neopentyl glycol	NPGDA	85
<u>-dimethacrylate</u>		
1,4-butanediol	BUDMA	95
1,6-hexanediol	HDDMA	95
ethylene glycol	EGDMA	98
triethylene glycol	TREGDMA	95
tetraethylene glycol	TEGDMA	95
neopentyl glycol	NPGDMA	c
<u>-triacrylate</u>		
pentaerythritol	PETA-3	c
oligo	OTA 480	c
trimethylolpropane	TMPTA	90
<u>-tetraacrylate</u>		
pentaerythritol	PETA-4	c
<u>Monofunctional</u>		
<u>-methacrylate</u>		
2-hydroxypropyl	2-HPMA	98

c = commercial grade.

The purity of PETA-3, PETA-4 and OTA 480 are discussed in paper VIII.

Induction procedure. The vehicle for intradermal injections was olive oil for some of the substances. As many of the acrylic compounds are rather viscous, they were dissolved in a mixture of olive oil and acetone (9:1) in order to get a better dispersion for intradermal injection. The Freund's Complete Adjuvant (CFA) used, was purchased from Difco Laboratories, Detroit, Mich., USA. 24 h. prior to topical application of the test substance, the area was pretreated with 10% sodium lauryl sulphate (SLS) in petrolatum without occlusion for the non irritant acrylic compounds. Even with SLS, some of the test substances were applied in a lower concentration than 100%, because they were too viscous to spread homogeneously over the test area. The final concentration and vehicle used for intradermal and topical induction are given in the various papers (II - IX).

Challenge. Two weeks after the topical application, the animals were tested with the commercial products and/or the pure substance. The patch test area was occluded for 24 h. and was read approximately 4 h. after the patches had been removed. The reactions were evaluated blind and an assistant chose the cages of the animals at random. If no animals or just a few animals showed positive test results, the GPM-test procedure was repeated once more and also with various induction concentrations.

Evaluation of challenge reactions. Two parameters were measured in estimating sensitization: The proportion of animals sensitized and intensity of the reaction.

The intensity of the reactions was evaluated according to the scale proposed by Magnusson & Kligman (45) with some modifications (47).

0	=	no visible change
0.5	=	very faint non confluent erythema
1.0	=	confluent erythema
1.5	=	erythema and slight edema
2.0	=	erythema and moderate edema
2.5	=	erythema and marked edema
3.0	=	intense erythema and marked edema

From this, the mean response is calculated by summation of the numerical readings of the challenge response from the various animals and dividing this sum by the total number of readings, including the negatives.

The proportion of animals sensitized was evaluated by counting the number of animals with only evident erythema and/or edema (≥ 1.0), which was regarded as an allergic response.

The test substances can be assigned according to the % of animals sensitized, to 1 of 5 classes, ranging from a weak (grade I) to an extreme (grade V) sensitizer (45).

Simultaneous reactions. In order to investigate cross-sensitivity (for the pure substances) or concomitant reactions, the animals were also tested with other chemically related substances and inhibitors or other chemicals, which might be present in the product. The animals were for the most of the acrylic compounds challenged twice with one week apart, and with a maximum of 6 test sites each time. In the first challenge the animals were tested on their right flank and in the second challenge the animals were tested on the left flank. 48 h. after the first challenge application the animals received a booster dose with the sensitizing chemical intradermally on the neck in the same concentration and vehicle as used for intradermal induction, but without (47).

All acrylates used for induction were also retested in the second challenge procedure. The chemicals used for challenge and their concentrations and vehicles are shown in the various papers (II - IX). The test vehicle for the test substances was petrolatum of an approximate quantity of 0.015 g. Most acrylic compounds were also tested with acetone as vehicle of a quantity of 0.02 ml and some chemicals with ethanol as vehicle of the same quantity.

In all challenge applications, Finn Chambers[®] (Epitest Ltd OY, Helsinki, Finland) were used.

Controls. At the same time as the animals in the experimental group were sensitized, approximately the same number of animals were also exposed intradermally to CFA and the same vehicle as for the experimental animals [olive oil or olive oil/acetone (9:1)] but not to the acrylic compounds. SLS-procedure was also used in the control animals as for the experimental group and with petrolatum for topical induction. When the sensitized animals were challenged, the control animals were patch tested with the same chemicals and at the same

concentrations. As a booster dose, the control animals received olive oil or olive oil/acetone (9:1) intradermally.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY - ANALYSIS (HPLC)

In order to check the purity of the acrylates tested, an isocratic high performance liquid chromatography (HPLC) system was used (Laboratory Data Control, LDC, Milton Roy Company, USA), comprising a LDC Constametric III pump fitted with Rheodyne loop injector and a variable UV-detector, LDC Spectro Monitor III. A Perkin Elmer 550 S UV/VIS spectrophotometer was used for determination of absorption maximum, in order to choose the optimal wavelength for UV-detection of the substances. For those acrylic compounds which were tested with their various fractions, a LKB 2112 Redirac fraction collector was used. The columns used for analysis, separation and isolation of the acrylates are described in detail in the various papers (V, VII, IX).

By using different columns and by detecting at various wavelengths, the purity of the substances was controlled. Different fractions were collected from repeated injections into the chromatograph. The purity of these fractions was then controlled by reinjecting them into the analytical column previously used. To some fractions, in order to prevent polymerization, an inhibitor (thymol) was added. From the fractions, the solvent was finally evaporated and the residue stored in a refrigerator.

NMR. For the di(meth)acrylates based on bisphenol A and/or epoxy resin, further analysis of the main fractions were performed with Nuclear Magnetic Resonance (NMR) Spectrometer at the University of Lund, Sweden, with a Jeol MH 100 Spectrometer (Jeol Ltd, Japan).

INFLUENCE OF THE TEST VEHICLE (IX)

The influence of the vehicle on the test response was performed on the prepolymers and multifunctional acrylates, except the methacrylated ones, with the GPM-test exactly in the same way as described above. The vehicle used for the various acrylic components for challenge was petrolatum and acetone. The two challenged tests were applied simultaneously on the guinea pig flank.

In animals sensitized to neopentyl glycol diacrylate (NPGDA) (Fig. 4), the influence of various vehicles (petrolatum, acetone and alcohol) and possible regional variations on the challenge response was investigated.

Influence of the vehicle. Chemical analysis with HPLC using NPGDA as a model for the acrylate monomer.

- A) The adsorption of the acrylic monomer to the aluminium chamber and/or a filterpaper disc was estimated.
- B) Possible polymerization of the monomer (with or without inhibitor) on the aluminium chamber was studied. Special interest was focused on the alteration of the amount of the residue monomer with increasing time.
- C) The influence the surface of various materials might have on a possible polymerization process was studied.
- D) A measure of a possible decrease of the monomer content with increasing time when petrolatum was used as vehicle was performed.

A detailed description of the chemical analysis is seen in paper IX.

RESULTS

THE PREPOLYMERS (III, IV, V, VII).

Polyester acrylates (IV). Of the 20 animals exposed to a polyester acrylate (Ebecryl 810), 33% became sensitized. Challenge with methyl methacrylate and methacrylate gave no reactions in the sensitized animals. The polyester acrylate tested was of commercial grade.

Di(meth)acrylates based on bisphenol A or epoxy resin (III, V). The epoxy acrylate and bisphenol A dimethacrylate (BIS-EMA) investigated in paper III were of commercial grade. When epoxy acrylate was used for induction, 90% of the animals exposed were sensitized and 45% of those also reacted to bisphenol A dimethacrylate (BIS-EMA). Rechallenge of the animals one week after a booster dose with epoxy acrylate, showed that all of the 20 animals became sensitized. 5 (25%) of the 20 animals reacted to epoxy oligomer MW 340, 1 to bisphenol A, and the same animal also to acrylic acid. No animals reacted to methyl methacrylate.

Bisphenol A dimethacrylate (BIS-EMA) (commercial grade) sensitized 40% of the animals. 70% also reacted to epoxy acrylate. After a booster dose, 45% of the

animals were sensitized. 30% also reacted to epoxy oligomer MW 340, 10% to acrylic acid, but none to bisphenol A and methyl methacrylate.

In paper V, 2 epoxy acrylates were tested, the Epoxy acrylate DOW and Epicote acrylate DRH 370. The commercial product of Epoxy acrylate DOW sensitized 53% of the animals in the first challenge. When tested with the main fraction 47% reacted. Test with the epoxy acrylate Epicote DRH-370, gave 13% positive animals. At the second challenge 93% of the animals became positive to the whole product, and also to the main fraction. One animal was also positive when tested with the commercial product of BIS-EMA (DIACRYL 101). No positive test reactions were seen for BIS-PMA, BIS-GMA, BIS-MA, epoxy resin MW 340, acrylic acid or hydroquinone. When sensitized with the main fraction of Epoxy acrylate DOW, 60% were positive in the first challenge and 93% in the second. Test with the whole product gave 93% positive animals. The commercial product of BIS-EMA (DIACRYL 101) gave positive test response in 20%. No animals reacted to BIS-PMA, BIS-GMA, BIS-MA and epoxy resin MW 340.

The challenge reactions in animals sensitized with the epoxy acrylate, Epicote acrylate DRH-370 gave 73% positive animals in the first challenge. All animals became sensitized in the second challenge to the whole product. All animals reacted also when challenged with Epoxy acrylate DOW and 8% with the main fraction of Epoxy acrylate DOW. Test with the whole product of BIS-GMA gave 20% positive reactions. No reactions at all were seen when tested with BIS-PMA, BIS-EMA (DIACRYL 101), the main fraction of BIS-GMA, BIS-MA, epoxy resin MW 340, acrylic acid and hydroquinone.

Three GPM-test procedures with BIS-PMA in various intradermal induction concentrations gave no positive animals.

Of those animals sensitized with the commercial product of BIS-EMA (DIACRYL 101), 33% reacted positively both in the first and the second challenge. 20% respectively 13% of the animals reacted to the main fraction of BIS-EMA. Test with the commercial product from another brand gave 20% positive animals. When tested with Epoxy acrylate DOW (main fraction), 13% became positive and 7% when tested with the whole product of Epicote acrylate DRH-370. No reactions were seen for BIS-PMA, BIS-GMA, BIS-MA and epoxy resin MW 340.

When the animals were sensitized with the main fraction of BIS-EMA (DIACRYL 101), 53 % were positive to the main fraction in the first challenge and 73% in the second. However, only 13% reacted to the whole product in challenge I and II. No animals reacted to BIS-EMA of another brand and none when tested with Epoxy acrylate DOW, Epicote acrylate DRH-370, BIS-PMA, BIS-GMA and BIS-MA.

Of the animals sensitized with the commercial product of BIS-GMA, 87% reacted when challenged with the whole product both in the first and the second challenge. Tests with the different fractions of BIS-GMA obtained by HPLC, showed that fraction ,1 (Fig. 8), gave 53% positive animals. The other fractions gave no reactions. Positive reactions in 87% of the animals to the whole product and 73% to the main fraction of Epoxy acrylate DOW, were observed. However, 14% of the animals also reacted to BIS-PMA. Challenge with epoxy resin MW 340, gave 80% positive reactions. Tests with BIS-EMA (DIACRYL 101), BIS-MA, methacrylic acid, triphenyl antimony, triphenylphosphine and hydroquinone were all negative.

Sensitization with BIS-MA (commercial product) shows that 53% and 67% of the animals reacted positively to the whole product of BIS-MA in the 2 challenge applications. Repeated GPM-test procedure gave 80% positive test (challenge III) to the whole product, and 93% to the main fraction and 47% animals reacted to the pre-fraction. When tested with epoxy resin MW 340, 27% reacted positively. No animals reacted to epoxy acrylate DOW, BIS-PMA, BIS-EMA (DIACRYL 101), BIS-GMA, bisphenol A, glycidyl methacrylate and hydroquinone. Animals sensitized to the main fraction of BIS-MA, showed that 80% reacted when tested with the main fraction of BIS-MA in the first challenge, and all animals reacted in the second challenge. When tested with the whole product, 47% became positive to this and 73% to the pre-fraction. No positive animals were seen when tested with Epoxy acrylate DOW, BIS-PMA, BIS-EMA (DIACRYL 101), BIS-GMA, bisphenol A, epoxy resin MW 340 and methacrylic acid.

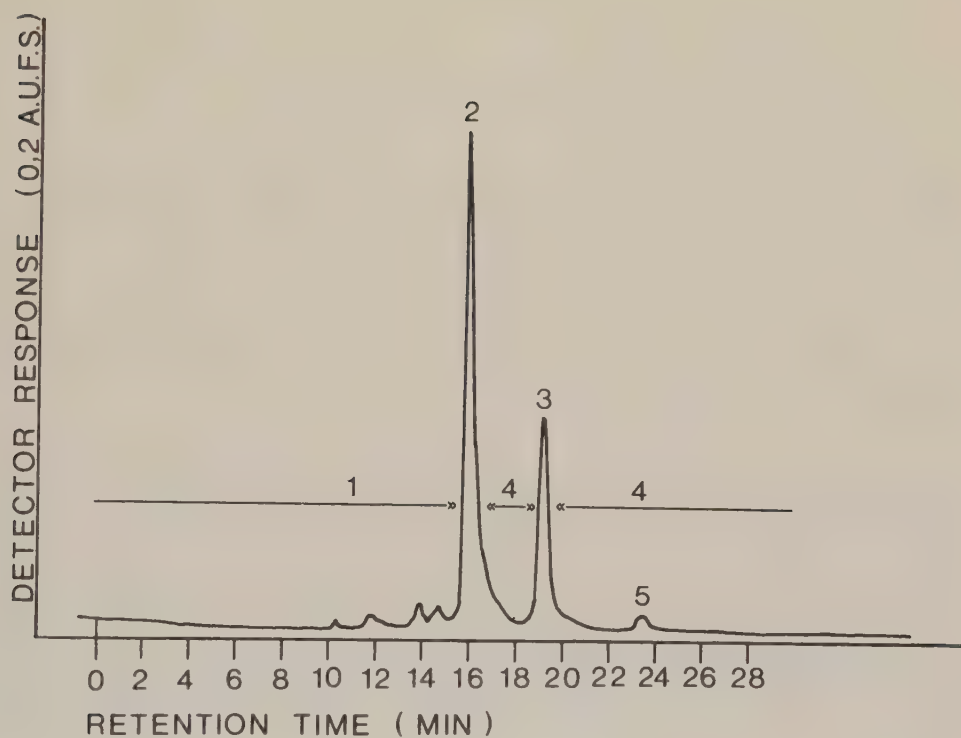


Fig. 8. Example of a chromatogram showing the separation of BIS-GMA (3 M) in different fractions by HPLC.
The identified peaks are: 2 = BIS-GMA, linear, 3 = BIS-GMA, branched, 5 = BIS-GMMA, bisphenol A diglycidyl monomethacrylate.

Urethane (meth)acrylates (VII). Three urethane (meth)acrylates were tested. Of those animals sensitized with the aromatic urethane acrylate (Ebecryl 220), 15% showed positive test reactions, Those guinea pigs sensitized with the aliphatic urethane acrylate (Ebecryl 270), a positive test response of 87% was found. Of those animals sensitized with the aliphatic urethane methacrylate (UEDMA), 15% reacted positively. None of the sensitized guinea pigs in the three different experimental groups reacted when challenged with the other two products. The purity of the urethane methacrylate was 98% and the urethane acrylates were of commercial grade.

THE MULTIFUNCTIONAL ACRYLATES (II, VIII).

Diacrylates (VIII). Of those animals sensitized with BUDA, 67% showed positive test reactions. Of those sensitized with HDDA, 60% were positive. 13% of the animals reacted positively when sensitized with DEGDA. None of the TREGDA and TEGDA sensitized animals reacted. Of those guinea pigs sensitized with TPGDA, 73% became allergic. All of the animals sensitized with NPGDA, gave a positive test response.

Di(meth)acrylates (VIII). Only 7% of the animals got allergic to TREGDMA. No sensitization was observed for the other di(meth)acrylates tested (BUDMA, HDDMA, EGDMA, TEGDMA and NPGDMA).

The simultaneous reaction pattern for the di(meth)acrylates tested is shown in Table 3. None of the animals sensitized to a diacrylate, reacted to any di(meth)acrylate.

Tri- and tetraacrylates (II, VIII). Of those guinea pigs sensitized with the commercial product of TMPTA (paper II), 67% became sensitized. 75% reacted when challenged with PETA. At the second challenge only 29% reacted to TMPTA. No animals reacted to TMPTMA and acrylic acid.

Of guinea pigs sensitized with the commercial PETA-3, 67% became sensitized. 6 of those also reacted to the "purified" PETA-3, 7 to the commercial PETA-4, 3 to the "purified" PETA-4, 7 to TMPTA and 3 to pentaerythritol.

Table 3. CHALLENGE REACTIONS AND SIMULTANEOUS REACTION PATTERN IN ANIMALS SENSITIZED WITH DI(METH)-ACRYLATES.

The figures indicate the positive animals of the animals tested. - = not tested.

Challenged with Sensitized to		Number of animals tested	DIACRYLATE								DI(METH)ACRYLATE							
			BUDA	HDDA	DEGDA	TREGDA	TEGDA	TPGDA	NPGDA	BUDMA	HDDMA	EGDMA	TREGDMA	TEGDMA	NPGDMA			
<u>Diacrylate</u>																		
	BUDA	15	10	7	3	-	0	1	0	0	-	0	-	0	-			
	HDDA	15	1	9	4	-	4	0	0	0	0	-	-	-	-			
	DEGDA	15	3	0	2	-	3	0	0	-	-	0	-	0	-			
	TREGDA	19	-	-	0	0	0	0	-	-	-	-	0	-	-			
	TEGDA	15	0	0	0	-	0	-	0	-	-	-	-	0	-			
	TPGDA	14	7	0	10	-	10	11	4	-	-	0	-	0	-			
	NPGDA	14	8	0	11	-	10	3	14	-	-	0	-	0	0			
<u>Di(meth)acrylate</u>																		
	BUDMA	10	0	0	-	-	0	-	-	0	-	0	-	0	-			
	HDDMA	15	0	0	0	-	0	0	-	0	0	-	-	0	-			
	EGDMA	13	0	0	-	-	0	-	-	0	-	0	-	0	-			
	TREGDMA	15	0	0	-	0	-	-	-	0	-	0	1	-	-			
	TEGDMA	15	-	-	-	-	0	-	-	-	-	0	0	0	-			
	NPGDMA	15	-	-	-	-	0	-	0	-	-	-	-	0	0			

No animals reacted to OTA 480 (paper VIII). Of those guinea pigs sensitized with OTA 480, 40% became positive. None of these reacted when challenged with PETA-3, PETA-4, TMPTA and trimethylolpropane.

Only 1 (7%) of the animals sensitized with the commercial product of PETA-4 reacted positively. The same animal also reacted to the "purified" products of PETA-4 and PETA-3 and to TMPTA. No animals reacted when challenged with OTA 480 and pentaerythritol. Challenge with hydroquinone gave no response in any of the animals tested. The simultaneous reaction pattern in animals sensitized with tri- and tetraacrylates are shown in Table 4.

MONOFUNCTIONAL ACRYLATES (VI)

Of those animals sensitized with 2-HPMA, 10% reacted positively. The same results (10%) were seen when tested with 2-HEMA. None of the animals reacted to 2-HPA, 2-HEA or hydroquinone.

HPLC-ANALYSIS

The purity of all the acrylic compounds tested has been investigated by HPLC-analysis. See Table 1 and 2.

The prepolymers (III, IV, V, VII) Polyester acrylate (IV). The polyester acrylate tested, Ebecryl 810, was not pure but of commercial grade.

Di(meth)acrylates based on bisphenol A or epoxy resin (III, V). The epoxy acrylate and bisphenol A dimethacrylate (BIS-EMA) investigated in paper III, were of commercial grade and impure.

Of those di(meth)acrylates based on bisphenol A or epoxy resin, investigated in paper V, only one product, BIS-PMA, was sufficiently pure. All the others showed various degrees of impurity. However, the main fractions were easy to recognize in all the acrylates and easy to collect for further testing. The chemical structures of all main fractions were controlled by NMR. The commercially used BIS-GMA could be resolved into 3 different components. By means of the NMR-method the corresponding peaks have been identified as

Table 4. CHALLENGE REACTIONS AND SIMULTANEOUS REACTION PATTERN IN ANIMALS SENSITIZED WITH TRI- AND TETRAACRYLATES

The number indicate the positive animals of 15 tested.

- = not tested

Challenged with Sensitized to	PETA-3		PETA-3 MAIN FRACTION		OTA 480	PETA-4	PETA-4 MAIN FRACTION	TMPTA	PENTA- ERYTHRITOL	TRI- METHYLOL- PROPANE
PETA-3	10	6			0	7	3	7	3	-
OTA 480	0	-			6	0	-	0	-	0
PETA-4	3	1			0	1	1	1	0	-

linear BIS-GMA and branched BIS-GMA (peak 2 and 3 in Fig. 8). NMR analysis also showed that the small peak (peak 5) in the HPLC-chromatogram represents the monomethacrylate derivative BIS-GMMA.

Urethane (meth)acrylates (VII). HPLC-analysis of the two urethane acrylates tested (Ebecryl 220 and Ebecryl 270) showed that both were of commercial grade. The chromatogram showed many peaks for both products suggesting impurities. The urethane methacrylate (UEDMA) had a purity of 98%.

Multifunctional acrylates (II, VIII).

TMPTA and PETA used in the investigation described in paper II, were commercial products and reported to be pure. HPLC-analysis showed that TMPTA and TMPTMA had a purity of at least 90%. The PETA contained some impurities and the main peak was identified as pentaerythritol triacrylate. The amount of pentaerythritol tetraacrylate was approximately 5%.

Most of the commercial di(meth)acrylates investigated in paper VIII, had a purity of at least 95% (Table 2). PETA-3 contained however high degree of impurities. One peak in the HPLC-chromatogram was identified as PETA-4. As the PETA-3 compound polymerized very easily, complete purification was impossible. The final "purified" product of PETA-3 contained approximately 1% of PETA-4. The commercial PETA-4 was purer than PETA-3 and the HPLC-chromatogram of the "purified" PETA-4, showed mainly 2 peaks. The smaller peak (approximately 15% of the main peak) was identified as PETA-3. As the PETA compound is a mixture of pentaerythritol tri- and tetraacrylate further purification was impossible. OTA 480 was also of commercial grade and impure.

Monofunctional acrylates (VI).

The purity of 2-HPMA, 2-HEMA, 2-HPA and 2-HEA investigated in paper VI was at least 95%.

THE INFLUENCE OF THE VEHICLE (IX)

GPM-test. The test results for the various acrylic compounds with acetone and petrolatum as vehicles are shown in Table 5.

The vehicles. A decrease in reactivity in animals sensitized to NPGDA was seen when tested not only in acetone but alcohol as well, compared to the petrolatum and the mixture of petrolatum and acetone (90/10) vehicle (Table 6).

Table 5. INFLUENCE OF THE VEHICLE ON THE TEST RESPONSE IN GUINEA PIGS SENSITIZED WITH VARIOUS ACRYLIC COMPOUNDS.

Acrylate used for induction		Number of positive animals of 15 tested	
		Petrolatum	Acetone
Prepolymers	Epoxy acrylate DOW	14	2
	Epoxy acrylate DRH-370	15	5
	BIS-PiMA	0	0
	BIS-EMA	5	0
	BIS-GMA	13	0
	BIS-MA	10	4
	Ebecryl 220	2	0
	Ebecryl 270	13	4
	UEDMA	2	0
Multifunctional acrylates	PETA-3	10	0
	PETA-4	1	0
	OTA 480	6	0
	BUDA	10	7
	HDDA	9	1
	DEGDA	2	0
	TEGDA	0	0
	TPGDA	11	0
	NPGDA	14	2

Table 6. INFLUENCE OF THE VEHICLE AND INHIBITOR ON THE ALTERATION OF MONOMER CONTENT ON AN ALUMINIUM CHAMBER AS A FUNCTION OF INCREASING TIME.

The numbers indicate % of initial concentration of NPGDA.

Vehicle & condition	Time (min.)				
	5	10	15	30	60
Acetone without inhibitor, daylight	91	84	60	16	8
Acetone with inhibitor, darkness	93	82	81	54	22
Acetonitrile without inhibitor, daylight	86	81	72	32	6
Acetonitrile with inhibitor, darkness	90	94	90	60	28

Table 7. INFLUENCE OF THE SURFACE ON THE ALTERATION OF MONOMER CONTENT AS A FUNCTION OF INCREASING TIME.

The numbers indicate % of initial concentration of NPGDA.

Surface	Time (min.)				
	5	10	15	30	60
Filterpaper	100	88	74	42	21
Glass	96	94	86	49	11
Aluminium	86	81	72	32	6

Test site location. When changing the patch test sites, half of the animals did not react when challenged with NPGDA in petrolatum near the abdomen compared to a test site close to the back (Fig. 9). No significant difference in the test response was observed with the acetone vehicle on the various test sites.

Adsorption. There was no indication that adsorption of the acrylic monomer to the aluminium surface or filterpaper disc occurs.

Polymerization. A marked difference in the monomer residue with increasing time, was seen between the acrylate solutions with (darkness) and without (daylight) inhibitor. The monomer content on the aluminium chamber when acetone was used as vehicle was approximately 8% of the initial amount after 1 h. (Fig.10).

The surface. The monomer decrease is more effected by the aluminium surface than the glass and filterpaper surfaces. 6% of the initial concentration of NPGDA was detected after 1 h. on the aluminium surface, compared to 21% on the filterpaper disc and 11% on the glass surface (Table 7).

Petrolatum. The monomer content is reduced to 62% of the initial amount of NPGDA after 2 h. After 24 h., 45% of the monomer remains.

DISCUSSION

In the last decade an increased number of workers are exposed to UV-curable acrylic compounds in a variety of industries. Skin problems in workers associated with acrylates in ultraviolet curing inks, varnishes or coatings have been reported in literature in recent years (6, 7, 10, 25, 26, 39, 48, 49, 52, 53, 64, 65, 98, 99).

Soon after changing from conventional printing inks to ultraviolet curing inks in 4 different printing plants in southern Sweden, 6 workers developed dermatitis on the forearms and face, as well as eye irritations (I). All the patients had a positive patch test reaction to the used ink and to TMPTA, the multifunctional acrylate present in the ink. 3 of the 6 patients also reacted to a polyester acrylate and 2 to an epoxy acrylate, both compounds present as prepolymers in the inks used. 4 men were positive to PETA. They had previously worked with

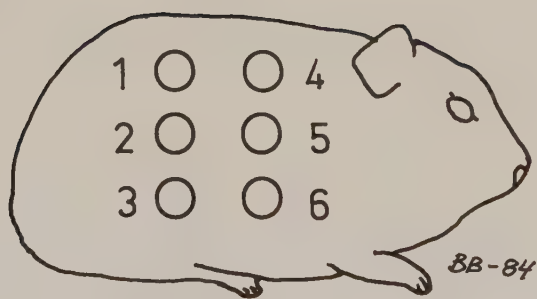


Fig. 9. Patch test location for challenge on the guinea pig flank.

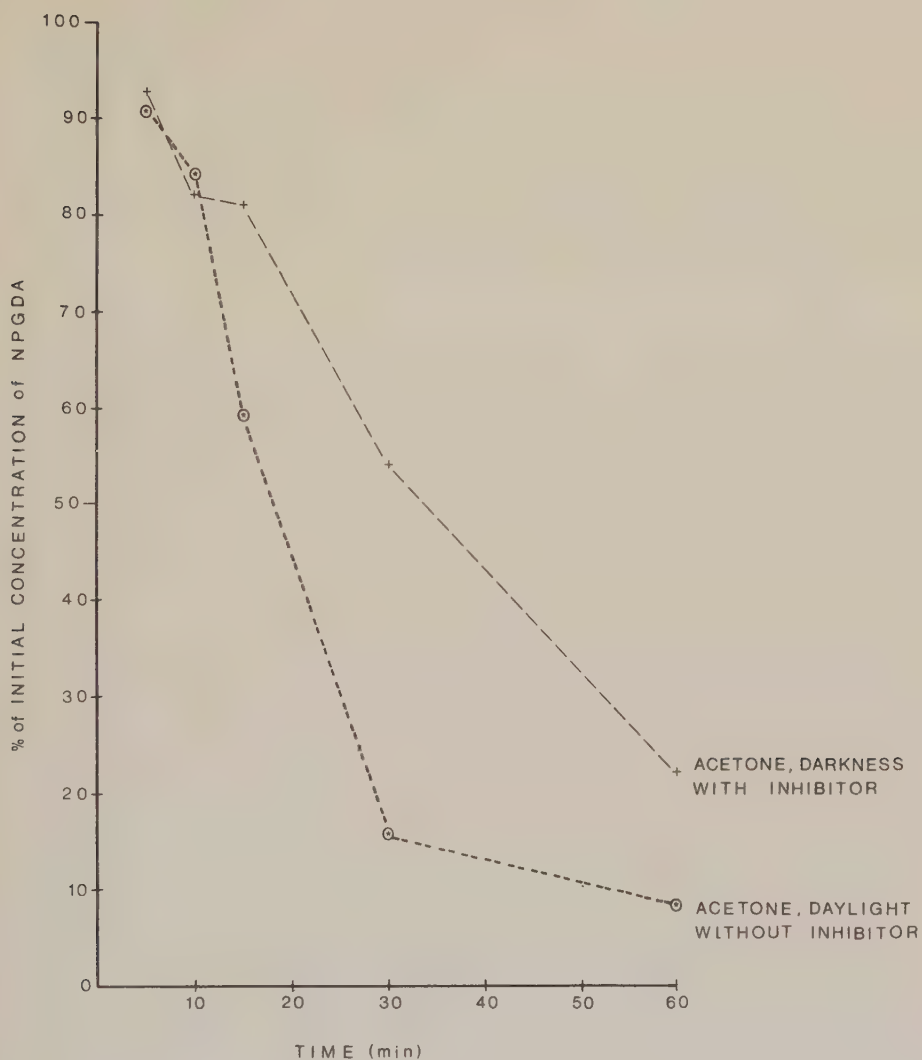


Fig. 10. The decrease of the NPGDA monomer on an aluminium chamber with time.

other ultraviolet curing inks, but information about the presence of PETA in these was impossible to obtain from the manufacturer. All the acrylic compounds tested were of commercial grade. Because of the high speed of the rollers of the printing presses, the inks form microdrops in the air. This air contamination might explain symptoms of itching on the face and forearms and the eye irritation. Those workers who reacted to the prepolymers also admitted that they did not use gloves when refilling inks and cleaning the printing press. Their hands and working clothes were often contaminated with inks and varnishes.

Presumably, many workers assume the inks are harmless and therefore fail to take appropriate precautions, because information from the manufacturerers has been given, that "allergy tests" has been performed and has shown that the inks are not "allergenic". Many serious ink producers devote a considerable amount of time and money to evaluate any hazards from accidental and repeated or prolonged exposure to radiation curable resins. In most cases, the primary skin irritation index, (classified according to the Draize system), the eye irritation index in rabbits, oral and inhalation toxicity in rats, and the mutagenic potential, using AMES test have been evaluated.

However, very few manufacturers have investigated, the possible allergic potential of their acrylic products. Usually, the producer as well as the consumer have no knowledge of the difference between irritant and allergic reactions. Therefore, the information given to the workers about possible skin hazards are often none or very scanty, and might even be false.

The increased incidents of allergic contact dermatitis due to UV-curable inks in a short time in the printing shops, together with the lack of information about their possible allergenic potential, initiated this investigation, concerning the sensitizing capacity of various acrylic compounds used in UV-curable resins.

THE PREPOLYMERS (III, IV, V, VII).

Polyesteracrylates (IV). 50% of the workers with dermatitis from UV-curing inks in the printing plants discussed earlier had positive test results from a polyester acrylate (Ebecryl 810), a common prepolymer in the inks used. No allergic contact dermatitis caused by unsaturated polyesters has previously been reported.

GPM-test with the polyester acrylate (Ebecryl 810) gave 33% positive animals, and the product is thus classified as a moderate sensitizer. The polyester acrylate tested was of commercial grade. It is thus unknown, if the whole molecular structure of the polyester acrylate acts as an allergen, or if the impurity is the sensitizer. No test reactions were seen with methyl methacrylate, methyl acrylate or with the proposed initiator hydroquinone. However, there are many various types of polyester acrylates and the GPM-test results found in this study are probably not valid for all of them. However, the reactive terminal acrylate groups, might be of great importance for antigen formation and sensitization.

Di(meth)acrylates based on bisphenol A or epoxy resin (III, V). Resins of the epoxy acrylate type for use as dental fillings have been described for more than 20 years (13). In spite of increasing use in the last decade of epoxy acrylates in other areas, like coatings or printing inks only a few reports about contact allergy have been published (8, 26, 64).

Of the 3 various brands of epoxy acrylate tested, 93% of the guinea pigs were sensitized to Epoxy acrylate DOW, all of the animals to Epikote acrylate DRH-370 (V) and 90% to the epoxy acrylate (G-MAN) investigated in paper III. GPM-tests performed by Nethercott (64) confirm these results. He investigated 6 brands of epoxy acrylates and found, that between 40% and 100% of the animals became sensitized to the products.

Of the 6 patients described previously (I), 2 had a positive patch test to epoxy acrylate (G-MAN), an ink constituent. None of the patients reacted to epoxy resin MW 340. The patient reported by Nethercott (64) was allergic to 3 of the 4 epoxy acrylates he had been working with, but not to epoxy resin MW 340.

Of the guinea pigs sensitized with epoxy acrylate (G-MAN) (III), 25% reacted positively to epoxy resin MW 340. Thin Layer Chromatography (TLC) showed presence of free epoxy resin in the epoxy acrylate.

After purification and testing with the main fraction of the Epoxy acrylate DOW (V), the same number of animals (93%) were positive as for the whole product. The chemical structure of the main fraction was controlled by NMR. No positive reactions were seen to epoxy resin MW 340 and acrylic acid, possible rests of

the manufacturing process. Nethercott (64) found the same results, as none of the animals sensitized to the six brands of epoxy acrylates, reacted to epoxy resin.

It is probably so, that the entire epoxy acrylate molecule has a sensitizing capacity of its own. However, if there are free epoxy resin present in the epoxy acrylates, there is also a potential risk for sensitization with an epoxy resin contaminant, when working with epoxy acrylates in UV-curing inks.

In repeated test procedures, sensitization with BIS-PMA was unsuccessful. Thus BIS-PMA seems to have a minimal or very low sensitizing potential. The BIS-PMA used for induction and challenge was pure. BIS-PMA is used in dental composite materials. There is no information if BIS-PMA is also used as a prepolymer in ultraviolet inks or coatings, but because of its low allergenic potential, it would be a good alternative to the other acrylates based on bisphenol A or epoxy resin, providing good technical properties.

Of those animals sensitized with a commercial product of BIS-EMA (DIACRYL 101), 1/3 of the animals became allergic. Induction with the main fraction of BIS-EMA, 3/4 of the animals reacted positively. This is in agreement with the findings in paper III, in which 40% of the guinea pigs exposed to bisphenol A dimethacrylate became sensitized. Bisphenol A dimethacrylate and BIS-EMA have the same chemical structure, but are of different brands (G-MAN resp. AKZO Chemie). None of the animals sensitized to BIS-EMA (DIACRYL 101) reacted to epoxy resin MW 340. However, 1/3 of those animals sensitized with bisphenol A dimethacrylate (G-MAN), reacted to epoxy oligomer MW 340. TLC showed no presence of epoxy resin MW 340 in bisphenol A dimethacrylate. The different findings cannot easily be explained at present.

No animals sensitized with the commercial product of BIS-EMA (DIACRYL 101) or its main fraction, reacted to Epoxy acrylate DOW. The bisphenol A dimethacrylate from G-MAN was of commercial grade and not pure. Some impurities present, might have allergenic potentials, and thus explain the difference in test results. However, the pure BIS-EMA (DIACRYL 101) is a moderate sensitizer and might cause allergic contact dermatitis when used as a monomer in dental resins and composite materials.

BIS-GMA is the resin matrix in all of the commercially available composite restorative materials used today (69). These materials can polymerize either chemically (e.g. benzoyl peroxide) or by exposure to UV-radiation together with an initiator. The major advantages of the UV-cured system is that it offers the dentist unlimited working time. There are some reports about contact allergic reactions to BIS-GMA. Nathanson & Lochart (61) described a patient with contact allergy to a composite dental material. They suggested that a possible sensitizer could be the BIS-GMA monomer in the composite matrix. Niinimäki *et al* (66) described a patient with perioral dermatitis after visiting the dentist. The patient had a positive patch test reaction to the filling material. BIS-GMA was also the main compound in the composite material used. Both patients also reacted to epoxy resin. The authors suggested that the positive test reactions to epoxy resin was due to a cross-allergy to BIS-GMA

During a period of a year, 702 patients with suspected contact allergy to dental materials, have been tested in various dermatology departments in Sweden with the "dental screening tray" (2). Two reacted to BIS-GMA and they were also positive to epoxy resin in the standard test series. 8 epoxy positive patients were also tested with BIS-GMA and 6 were positive (11).

BIS-GMA can be resolved into 3 different components by qualitative HPLC-analysis. By means of NMR, 2 of the components have been identified in paper V, and by others (75, 95) as a linear BIS-GMA and a branched BIS-GMA (peak 2 and 3 in Fig. 8). The small peak (peak 5) in the HPLC-chromatogram represents the monomethacrylate derivative BIS-GMMA, which also was confirmed with massspectrometry (75). A fourth isomer, the double branched BIS-GMA should also be expected in the mixture in a quantity of about 2% (75). This isomer has not been detected in the chromatogram in this study. In all BIS-GMA products analysed, it is always the same proportion (3:1) of linear and branched isomers (75). The branched BIS-GMA isomer is not intentionally added to the monomer mixture, but it should be considered a side product in the synthesis of the BIS-GMA monomer (95). BIS-GMA can be synthesized in two ways: 1) by the reaction of glycidyl methacrylate with bisphenol A, 2) by the reaction of the methacrylic acid with diglycidyl ether of bisphenol A (epoxy resin). The reaction of the 2 compounds is in either way, accompanied by a ring opening of the epoxide group. Both epoxide moieties of the molecule react independently from each other, and therefore the different BIS-GMA isomers are formed (Fig. 1).

Of those guinea pigs sensitized to the commercial product of BIS-GMA (3 M), 87% reacted positively when tested with the whole product. Only fraction 1 (free from linear and branched BIS-GMA and BIS-GMMA), (Fig. 8), of the different fractions of BIS-GMA tested, gave positive reactions in 53% of the animals. Thus, the linear and branched isomer or BIS-GMMA (fraction 4) did not seem to have any allergenic properties. The challenge procedure was even repeated with various test concentrations. 80% of the animals reacted positively to epoxy resin MW 340. Thus, it seems as if only the impurity fraction (fraction 1) of the BIS-GMA product possesses allergenic potential. In the BIS-GMA chromatogram, the epoxy resin MW 340 has its peak in the same area as fraction 1. It is uncertain if any residual epoxy resin monomer is left unreacted or formed in the synthesis of the BIS-GMA monomer. The presence of epoxy resin MW 340 is a probable explanation to the high number of positive epoxy tests in the BIS-GMA sensitized animals as well as in humans.

Compounds such as methacrylic acid, are known to be present in BIS-GMA resins, primarily because of an incomplete synthesis reaction. Traces of compounds such as triphenyl antimony and triphenylphosphine, or their oxidized derivatives, may also be present (14). Tests with methacrylic acid, triphenyl antimony and triphenylphosphine and hydroquinone were all negative.

Induction with the commercial product of BIS-MA, gave up to 80% positive animals to the whole product, 93% to the main fraction and 47% to the impurity fraction (pre-fraction). Induction with the main fraction of BIS-MA sensitized all animals. Test with the whole product gave 47% positive animals and with the impurity fraction as many as 53%. The impurity fraction was free from the main fraction. Thus, it seems that not only the main fraction but also the impurity fraction (pre-fraction), contains an allergen and cross-reaction between the two substances occurs. Of the animals sensitized to the whole product of BIS-MA, 27% reacted to epoxy resin MW 340. However, none of the animals sensitized with the main fraction of BIS-MA reacted to epoxy resin MW 340. It is probable, that small amounts of any epoxy resin residual present in the pre-fraction of the BIS-MA product, sensitized some animals. These reactions are not explained by the presence of bisphenol A, glycidyl methacrylate or hydroquinone, which all gave negative reactions.

The simultaneous reaction pattern between the di(meth)acrylates based on bisphenol A or epoxy resin are shown in Table 8. It is not unlikely, that a component in the impurity part of the commercial BIS-EMA (DIACRYL 101) which are structurally related to the epoxy acrylate molecule, occurs in a concentration efficient to sensitize some animals.

It also seems as if a concomitant reaction occurs between the commercial product of BIS-GMA and the main fraction of Epoxy acrylate DOW. However, the BIS-GMA sensitized animals only reacted to fraction 1. It is probable, that fraction 1 of the BIS-GMA product, contains small amounts of epoxy acrylate and similar substances, which can cross-react with each other.

The investigation in paper V has shown, that epoxy acrylate, BIS-EMA and BIS-MA are strong sensitizers, while the linear fraction of BIS-GMA and its isomers and BIS-PMA are weak or non sensitizers. The chemical structure of the linear BIS-GMA is that of an epoxy methacrylate. With the introduction of a methyl group at the α -carbon atom in the terminal acrylic part, the sensitizing potential is decreased. This has been shown in other investigations (9, 71, 92). However, in spite of the methacrylated end-groups, BIS-EMA and BIS-MA are strong sensitizers. BIS-PMA did not sensitize any animals. The BIS-PMA molecule has a linear structure with 3 methylene-groups in each moiety. Thus, it seems as 3 or more methylene-groups in the molecular chain diminish the allergenicity. The impurities in the BIS-GMA and BIS-MA batches seem to have high allergenic potential.

Urethane (meth)acrylates (VII). The acrylated urethanes are used as prepolymers in UV-curable coatings, but as resins for dental applications as well. The acrylated urethanes used in dentistry are mostly of the methacrylated types. The urethane methacrylate plays the same role as the more common BIS-GMA in composite and sealant formulations (75).

The most common of the urethane methacrylates used in dental resins, are of the aliphatic types based on bis-ethylene glycol methacrylate (95). The proposed general structure of the urethane methacrylate (UEDMA) investigated in paper VII,

Table 8. SIMULTANEOUS REACTION PATTERN IN ANIMALS SENSITIZED AND CHALLENGED WITH ACRYLATES BASED ON BISPHENOL A OR EPOXY RESIN

Challenged with Sensitized to	Epoxy acrylate DOW	BIS-PMA ^a	BIS-EMA (DIACRYL 101)	BIS-GMA ^a	BIS-MA
Epoxy acrylate DOW	14	0 ^a	3 ^a	0	0 ^a
BIS-PMA ^a	0	0 ^a	0	0	0 ^a
BIS-EMA (DIACRYL 101)	0	0 ^a	11	0 ^a	0 ^a
BIS-GMA ^a	11	2 ^a	0 ^a	13 ^a	0 ^a
BIS-MA	0	0 ^a	0 ^a	0	15

The number indicates the maximum number of positive animals.

^aInduction or challenge performed with the whole product, otherwise with the main fraction of the acrylate.

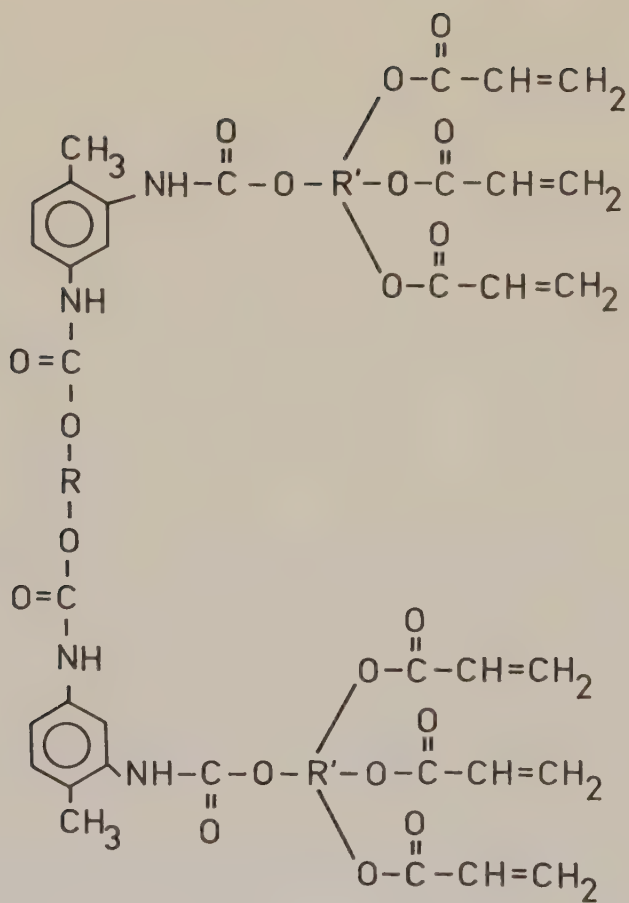
is shown in Fig. 2. The aliphatic chain is probably a mixture of several isomers.

Reports about contact allergy from urethane (meth)acrylates have been sparse. Three cases of contact hypersensitivity from anaerobic sealants are reported by Dempsey (24). The allergen was believed to be a substance labelled "polyurethane dimethacrylate". Nethercott et al (65) reported that several workers exposed to ultraviolet printing inks developed contact dermatitis. An urethane acrylate resin accounted for 5 of the cases. One of the patients was also sensitive to PETA, and another to epoxy acrylate resin. Nethercott et al also performed GPM-test with the urethane acrylate. They succeeded in sensitizing all animals with the highest intradermal induction concentration used.

Of the two commercial urethane acrylates tested in paper VII, the aliphatic one (Ebecryl 270) seems to be a more potent sensitizer than the aromatic one (Ebecryl 220). Ebecryl 220 is based on toluene diisocyanate (TDI) with the molecular weight of 1000. Ebecryl 270 has a higher molecular weight (1500). The type of aliphatic isocyanate used to synthesize Ebecryl 270, was unknown, but 1,6-hexamethylene diisocyanate (HDI) might be a possible isocyanate moiety. Ebecryl 220 has 6 functional groups (Fig. 11), while Ebecryl 270 only has 2. It is not known if the functional groups attached to the oligomer are of importance for the allergenicity of the compounds. The two urethane acrylates were of commercial grade and impure. Therefore, it cannot be excluded that any impurities left unreacted, formed or added during the synthesis of the final product, might possess allergenic potentials. Which, or what kind of impurity in the product that might be responsible for the allergenicity, is impossible to know without further purification and tests.

Thus, if an aliphatic urethane acrylate, in general, is a stronger sensitizer than the aromatic one, is unpredictable without further investigations. Analysis performed with HPLC on the aliphatic urethane methacrylate (UEDMA), showed a 98% purity. The molecular weight of UEDMA is 470 and the molecule has 2 functional groups. It is believed that UEDMA itself seems to possess allergenic potential as a mild sensitizer.

There are many various types of urethane (meth)acrylates on the market, used for both industrial, as well as dental purposes. One urethane acrylate can be a more potent sensitizer than another, depending on the molecular weight, acrylic or methacrylic functional groups, and last but not least, the influence of the impurity.



EBECRYL 220

Fig. 11. The proposed formulae of Ebecryl 220 where R might be an ethylene group and R' a substituted pentaerythritol.

In the last decade, quite a few reports about contact allergy caused by various multifunctional acrylic compounds have been published (6, 8, 19, 25, 26, 48, 51, 53, 62, 81, 98, 99). A peculiar type of delayed irritant contact dermatitis from HDDA and BUDA, has been reported by Malten et al (50). The HDDA and BUDA, tested in paper VIII, are moderate to strong sensitizers. The sensitizing potential of BUDA was questionable in a study made by van der Walle et al (94). They also failed to demonstrate a sensitizing potential of HDDA in a GPM-test. However, HDDA appeared to be a sensitizer in a Freund's Complete Adjuvants Test (FCAT). Nethercott (63) as well as Parker & Turk (71) succeeded in sensitizing guinea pigs to HDDA. Parker & Turk (71) were unable to induce contact sensitivity to acrylic compounds, that were substituted with the methyl group on the α -carbon atom (carbon 2) in the acrylic moiety. This is in agreement with results found in this investigation, where most of the dimethacrylates tested were non-sensitizers.

TREGDA as well as TEGDA was found to be non-sensitizers. Cavelier et al (18) found however, TREGDA and TEGDA to be moderate sensitizers and they also observed cross-reaction between the two diacrylates. Cavelier et al (18) studied the sensitization potential of various n-ethylene glycol di-acrylates and dimethacrylates in the GPM-test. They also found that the dimethacrylates, EGDMA, DEGDMA, TREGDMA and TEGDMA were sensitizers. This is not in agreement with the findings in this study or from results achieved by Parker & Turk (71).

Five patients with positive reactions to EGDMA and 2 to TREGDMA, were found among 702 patients tested with dental materials at various Dermatology Departments in Sweden, during a year (12).

Two of 4 patients described by Malten & Bende (51) had positive patch test reactions to DEGDMA and TEGDMA. One patient also reacted to EGDMA.

Bullous irritant reactions in workers at a large newspaper plant manufacturing printing plates, using photopolymer acrylic resins, were observed by Beurey et al (16). The irritant reactions were described to the irritant effect of TEGDA. Replacing the acrylate with the corresponding dimethacrylate (TEGDMA) eliminated the problem.

A patient working with pressure sensitive adhesive showed sensitivity to TEGDA (98). The patient was also patch test positive to HDDA, a non work-related acrylate.

TPGDA and NPGDA tested in this investigation, were found to be strong to extreme sensitizers. However, both acrylates were impure, and thus, it is uncertain if the proper allergens are the acrylic monomers or its impurities.

In UV-curable ink or coating formulations, PETA, TMPTA or HDDA are the multifunctional acrylates, used most commonly.

PETA is a mixture of pentaerythritol tri- and tetraacrylate, and the PETA-3 used in this investigation consisted according to the manufacturer, mostly of pentaerythritol triacrylate. PETA-3 seems to be a much stronger sensitizer than PETA-4. Simultaneous reactions were also seen between PETA-3 and PETA-4. Some PETA-3 sensitized animals also reacted to pentaerythritol. Animals sensitized to PETA-3 and PETA-4 also reacted to TMPTA. There is a possible cross-reactivity between PETA and TMPTA.

The fact that PETA is a potent sensitizer in guinea pigs has also been reported by other investigators (62, 64, 65, 71).

Contact allergy to PETA has also been described in humans (25, 26, 62, 81 99). Most of the patients described, sensitized to PETA, also reacted to TMPTA. The authors could however, not determine whether the reactivity was a result of cross-sensitization or concomitant sensitization, as most subjects had potential exposure to both PETA and TMPTA.

All 6 patients with dermatitis after working with UV-curing inks at printing plants in southern Sweden, described in paper I, reacted to TMPTA, the multifunctional acrylate present in the inks used. Four of the 6 men, also reacted to PETA, not present in the actual inks. However, those workers had worked with other UV-curable inks previously, which might contain PETA. None of the patients however, reacted to trimethylolpropane trimethacrylate (TMPTMA). GPM-tests (II) confirm, that there is no cross-reaction between TMPTA and TMPTMA. 67% of the guinea pigs were sensitized to TMPTA, which therefore can be classified as a strong sensitizer (II). Of the TMPTA sensitive animals, 75% also reacted to PETA, suggesting cross-sensitivity between the two substances. However, the PETA used for testing, is a mixture of pentaerythritol

triacrylate and tetraacrylate, and not completely pure.

TMPTA has been found to be used in other areas than UV-curable resins (19). Four workers developed dermatitis when exposed to a floor top coat. TMPTA was used to synthesize a polyfunctional aziridine hardener. Two of the four workers also reacted to PETA.

The oligotriacrylate OTA 480, which can be used as a substitute for the other triacrylates is a moderate sensitizer. The OTA 480 tested was of commercial grade.

The investigations demonstrate, that of the multifunctional acrylic monomers, used on the market, the di- and triacrylic compounds should, with some exceptions, be regarded as relatively potent sensitizers. The methacrylated multifunctional acrylates are weak or non sensitizers.

THE MONOFUNCTIONAL ACRYLATES (VI).

The monofunctional acrylic monomers used in UV-curable resins, are included in the coating to facilitate the application and for their contribution to the properties of the cured coating. They are also valuable viscosity reducers and augment the cross-linking density. The reactive monomers reported in literature are all members of the vinyl monomer family (80). Among others a common monofunctional acrylic monomer used in UV-curable resins are 2-hydroxypropyl methacrylate (2-HPMA). Apart from being a common monomer in UV-curable resin formulations, 2-HPMA is also used in ultraviolet sensitive compositions for fissure sealants in dentistry (73). Of the acrylic monomers, preferably used in UV-curable coatings and inks or in the photoprepolymer printing plate procedure, are hydroxyethyl acrylate, hydroxypropyl acrylate, hydroxyethyl methacrylate and ethylhexyl acrylate. The sensitizing potential of many mono(meth)acrylates has been thoroughly investigated by various authors in guinea pigs.(10, 17, 36, 51, 92, 98).

The investigation shows, that 2-HPMA is a weak sensitizer in guinea pigs. The data suggest cross-reactivity or concomitant reactivity to 2-HEMA. There are reports on positive patch test reactions to 2-HPMA in printers exposed to NAPP[®] printing plates (3, 4, 90). One patient, allergic to hydroxypropyl methacrylate, among 45 patients with shoe dermatitis, is described by Grimalt & Romaquera (29).

Contact sensitization to 2-HEMA has been reported. Malten & Bende (51) described 4 of 5 patients positive to 2-HEMA, one of the ingredients in a photoprepolymer mixture.

Parker & Turk (71) could not sensitize guinea pigs to 2-HEMA. Van der Walle et al (92) could however, sensitize all guinea pigs tested, with 2-HEMA.

Contact allergy to 2-ethylhexyl acrylate (2-EHA) has also been reported. Five of 7 patients with allergic contact dermatitis from an acrylic based adhesive tape, showed positive test results to 2-EHA, one of the ingredients in the adhesive tape (36). They were all negative to 2-ethylhexyl methacrylate. Two of the subjects were also positive to 1-HPA and 1-HPMA. Waegemaekers & van der Walle (89) found 2-EHA to be a strong sensitizer in guinea pigs tested with Freund's Complete Adjuvant Test.

Only 1 animal out of 10, became sensitized to 2-HPMA in the study described in paper VI. That animal did not react to 2-HPA and 2-HEA.

INFLUENCE OF THE TEST VEHICLE (IX)

Many factors have influence on the test response in sensitized guinea pigs. The effect the vehicle may have on the elicitation of hypersensitivity, is probably the most important one. A great discrepancy between the test results for various acrylic compounds, is seen when petrolatum and acetone are used as test vehicles. The number of positive animals, as well as the mean response figures, are markedly decreased, when the various acrylates were tested in acetone. If only acetone had been used as test vehicle, quite a few of the acrylates tested, would even had been considered as non-sensitizers. The intensity of the challenge response was also greatly improved by using petrolatum instead of acetone. Among the factors that might effect the challenge response are chemical reactions between the allergen and the vehicle, polymerization of the test substance, penetration, evaporation, adsorption of the allergen to the filterpaper or aluminium disc, interference of simultaneous skin test reactions and patch test locations. The number of positive guinea pigs sensitized with various acrylic compounds with petrolatum and acetone as vehicle, are shown in Table 5. By using neopentyl glycol diacrylate (NPGDA) as an acrylic monomer model, the same decrease in test response was seen not

only with acetone, but with alcohol as well, as vehicle compared to petrolatum (Table 6).

By changing the test site location (Fig. 9), half of the animals did not react when challenge test was applied near the abdomen, compared to a patch test site close to the back, with petrolatum as vehicle.

The chemical structure of vinyl ester resin is shown in Fig. 12. The acrylic or methacrylic moiety and the two ester linkages, are considered to be the point of greatest vulnerability to chemical attack. The ether linkages and the methyl-groups (shown as R), are considered to improve chemical resistance. Organic solvents can easily form peroxides, as well as acrylic compounds containing ethylene ether groups $[(CH_2CH_2O)_n]$, because of the lack of oxygen atoms in the backbone (26). With acetone, for example, the radical formed, can initiate polymerization of the acrylic compounds. Acetone has previously been used as a photoinitiator for certain polymerization processes (67).

The amount of acrylic monomer (NPGDA) in the test solutions, was found to decrease with increasing time. There was also a marked difference in the monomer residue between solutions with (darkness) and without (daylight) inhibitor. The monomer content on the aluminium chamber when acetone was used as vehicle, was approximately 8% of the initial amount after 1 h. The monomer decrease was also more effected by an aluminium surface, than a glass or filterpaper surface. This might be explained by the fact, that aluminium oxide, always present on an aluminium surface, is a catalyst (35), and could therefore enhances probable polymerization process.

With the petrolatum vehicle, the monomer content is reduced to a 2/3 after 2 h. After 24 h. about half of the monomer remains. Because the availability of the acrylic monomer in the petrolatum vehicle is only reduced by half at the test site after 24 h., the high challenge response could in part be explained.

Adsorption of the acrylic monomers to the aluminium surface or filterpaper disc has been shown not to occur. Thus an adsorption effect, cannot explain the discrepancy in the test results.

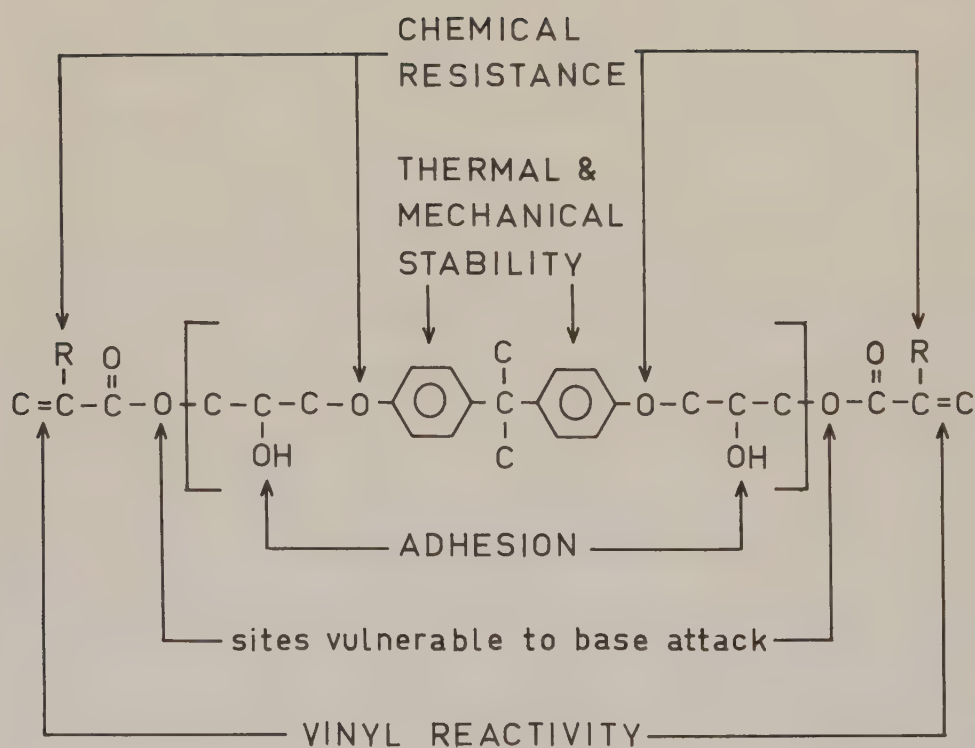


Fig. 12. Chemical resistance of a vinyl ester resin structure adapted from Carey & Launikitis (22).

The investigations in paper IX show that the discrepancy between the test results, when petrolatum and acetone was used as test vehicle, is most certainly due to a polymerization process of the acrylic compounds. Thus, the petrolatum vehicle probably prevents the acrylic monomer to polymerize.

INHIBITORS

In any formulations involving free radical chemistry, inhibitors or stabilizers are important ingredients. They retard or prevent polymerization until cure is desired. From the information obtained from the manufacturers of acrylic compounds, hydroquinone and its derivative, were the inhibitors mostly used. p-Methoxyphenol is also a common inhibitor.

There are some reports about patch testing with hydroquinone in acrylate sensitive patients. Magnusson (43) sensitized a few patients to methyl methacrylate. Two of them had also been sensitized to hydroquinone used in the monomer. Magnusson also noted that 5 of 8 volunteers, exposed to hydroquinone-stabilized methyl methacrylate monomer, became hypersensitive to hydroquinone. The author suggested that methyl methacrylate somehow facilitates or predisposes to the development of the hydroquinone hypersensitivity. Magnusson & Mobacken (46) found that none of those workers sensitized to an acrylic sealant, were allergic to hydroquinone. Kaaber & Thulin (37) described a patient with allergy to acrylic dentures. The patient was also positive to hydroquinone. Van der Walle et al (91, 92, 94) analysed the sensitizing potential of mono(meth)acrylates and di(meth)acrylates in guinea pigs. They observed concomitant sensitization to hydroquinone and/or p-methoxyphenol present in the acrylic monomers. The inhibitors had no influence on the sensitizing potential of the acrylic monomer (93). Induction and challenge with the inhibitors showed hydroquinone and p-methoxyphenol to be moderate sensitizers and cross-reactions between the two compounds occurred (91). None of the animals sensitized to the various commercial acrylate products described in the various papers (II - VIII), were found to be allergic to hydroquinone. The challenge concentration of hydroquinone was 0.1% w/w in ethanol. A concentration of 0.5% and higher was found to be irritating. Van der Walle et al (91) used a challenge concentration of 1 M (approximately 10%) in Aramek (2 parts methyl ethyl ketone + 1 part arachis oil v/v). A too high concentration, and thus false positive tests, might explain the different results between our investigations and theirs. As cross-reactivity between hydroquinone and p-methoxyphenol occurs (91) challenge with hydroquinone was only performed.

Thymol has been added to some purified fractions of acrylic compounds to prevent polymerization. Test with thymol have not been performed, as in various animal experiments, no sensitizing potential of thymol has been detected (38, 91).

THE PURITY

Cross-sensitivity implies, that a secondary allergen chemically related to the primary allergen, can provoke an allergic contact dermatitis or cross-reaction at patch testing. When discussing cross-reactivity or cross-sensitivity, it is important to know, that the primary and secondary allergens are pure. A chemical can not be classified as an allergen, unless the pure substance is used both for induction and challenge. In many of the acrylic compounds tested, a higher level of purity was difficult to obtain, because unwanted polymerization reactions interfered with the purification procedures. Therefore, sensitization to impurities can not be excluded, in particular not in tests where the acrylic compounds have been used in high concentrations for induction and challenge. However, in most test procedures, the concentrations used for intradermal injection and challenge are very low. Positive reactions caused by impurities are in most cases unlikely. A component in the impurity part of an acrylic compound used for induction, can be structurally related to another acrylate used for challenge, and thus, give a false interpretation about cross-reactivity. This fact emphasizes the importance of a pure substance, when discussing allergenicity and cross-reaction.

PREVENTION

Most of the acrylic compounds used in UV-curable resins, should be regarded as relatively potent sensitizers and care should be taken accordingly to minimize their contact with the skin in the course of their industrial use. The introduction of UV-curing resins in printing inks has thus, necessitated a complete re-consideration of the handling of such inks in the printing shops, and of the preventive measures that have to be taken in order to protect workers at plants and printing shops. Most of the acrylic resin manufacturers, when discussing skin hazards, are mostly concerned about the skin irritation their products might cause. That the acrylates can cause allergic reactions, are seldom discussed or mentioned in their safety data sheets. Predictive animal tests ought to be performed by the manufacturer for each acrylic compound produced, and the results clearly explained to the consumer on the data sheets.

From most of the producers of acrylic compounds, there are great difficulties in getting proper information about chemistry, purity, chemical structure, quantity of rest monomers, inhibitors etc., important information when discussing the sensitizing capacity. It is also a very valuable information to the doctor to know, if contaminants and what kind of contaminants, have been added or formed during the production process, when investigating the cause of a dermatitis.

The chemical nomenclature is also very confusing in the prepolymer and plastic area, not only for consumers, but for chemists working in the field as well. Thus, a data sheet of a product can be misleading and even false.

Although, no absolute relationship can be established between molecular structure and skin irritation, both the producer and consumer of acrylic compounds, have observed that skin irritation will be higher with an increasing number of acrylic double bonds, with decreasing viscosity and the presence of hydrophilic groups (83). Some producers also inform the consumers, that, the smaller the molecule of the acrylic monomer, the more toxic the compound, and the larger the molecule, the larger the sensitizing properties.

The Society of British Printing Ink Manufacturers, has advised their members that certain acrylic compounds, because they might cause skin irritation, should not be included in UV-ink formulations. Among those acrylates investigated here, which are to be avoided are: 2-HEA, 2-HPA, HDDA, NPGDA, 2-EHA, DEGDA and mixtures of pentaerythritol tri- and tetraacrylates (PETA). Apart from being a skin irritant and a strong sensitizer, NPGDA is an experimental tumorigen and probably an experimental carcinogen (82).

Thus, handling the UV-curable resins requires cautions. The prepolymers and multifunctional acrylates should be handled in the same way as has been known to acrylate monomers. Generally, the acrylate monomers are more volatile than the multifunctional acrylates and the more viscous prepolymers. Because of the high speed of the rollers of the printing presses, the inks form microdrops in the air. This air contamination might explain symptoms of itching on the exposed body areas and irritation of the eyes.

Safe handling of UV-curable acrylic resins can be possible by following the basic procedures suggested below:

- Wear protective clothing.
- Face shields are recommended wherever there is a risk of liquid spilling, spattering or misting.
- Tightly-fitting pair of safety goggles are recommended.
- Do not wear contact lenses, they might deteriorate.
- Wear gloves all the time.
- Do not use leather, cloth, PVC or any other natural rubber gloves.
- Neoprene, nitrile rubber, polyethylene or polypropylene gloves are recommended.
- In case of contact with the skin, wipe off and wash with plenty of water and soap.
- Prompt removal of contaminated clothing.
- Separation of clean and contaminated clothing.
- Avoid spilling. In case of spillage or leaks, absorb the liquid in clay or sundust. The spillage area should be thoroughly cleaned with soap and water.
- An effective vapor exhaust and ventilation system must be used.
- Do not smoke or eat in areas where chemicals are handled or stored.

By following these guidelines, together with education of the employees, the skin hazards can be reduced. If the manufacturers, also are willing to develop and produce resins of preferably the methacrylated types, with high degree of purity, the skin problems might even be avoided.

GENERAL SUMMARY

UV-curable acrylic compounds contain 3 basic components: a reactive base prepolymer, a diluent system composed of multifunctional acrylic esters (and, at times, monofunctional acrylic esters) and a photoinitiator system. The prepolymer is a synthetic resin into which the very reactive acrylate or methacrylate groups have been introduced. The commonly used prepolymers in the UV-curing field are among others: acrylated epoxies, acrylated urethanes and acrylated polyesters. Acrylates based on bisphenol A or epoxy resin are other prepolymers used not only in industrial applications, but in dental composite restorative materials as well.

The multifunctional acrylates are for example di(meth)acrylate esters of dialcohols or tri- and tetraacrylate esters of polyalcohols. The most commonly used multifunctional acrylate in UV-curable ink or coating formulations, is an acrylic acid ester of either pentaerythritol (PETA), trimethylolpropane (TMPTA) or hexanediol (HDDA).

Some commonly used monofunctional acrylates in UV-curable resin formulations are 2-EHA, 2-HPA, 2-HEA, 2-HPMA and 2-HEMA.

In 4 printing plants, 6 workers developed dermatitis (I). All had positive patch tests to the used ink and to TMPTA, the multifunctional acrylate present in the inks. Three workers also reacted to a polyester acrylate and 2 to an epoxy acrylate, both present as prepolymers in the inks. Four men also reacted to PETA not present in the inks used.

The fact that these 6 cases of allergic contact dermatitis occurred within a short period of time and the incidence of similar cases reported in the literature, increasing use of UV-curable resins and the lack of information about their possible allergenic potential, initiated this investigation.

The sensitizing capacity of various acrylic compounds was investigated using "The Guinea Pig Maximization Test" (GPM-test).

The polyester acrylate (IV) (Ebecryl 810) tested was classified as a moderate sensitizer.

Of the di(meth)acrylates based on bisphenol A or epoxy resin (III, V), the epoxy acrylate, BIS-EMA and BIS-MA are strong sensitizers while BIS-GMA (linear fraction and its isomers) and BIS-PMA are weak or non sensitizers. It seems as 3 or more methylene - groups in the molecular chain diminish the allergenicity. The impurities in the BIS-GMA and BIS-MA batches have a high allergenic potential.

Of the two commercial urethane acrylates (VII) tested, the aliphatic one (Ebecryl 270) seems to be a more potent sensitizer than the aromatic one (Ebecryl 220). The pure aliphatic urethane methacrylate (UEDMA) is a mild sensitizer. The urethane acrylates are impure, therefore it is not certain that an aliphatic urethane acrylate, in general, is a stronger sensitizer than the aromatic one or an methacrylated aliphatic urethane.

The sensitizing capacity of various multifunctional acrylates and their simultaneous reaction pattern have been investigated in paper III and VIII. BUDA and HDDA are moderate to strong sensitizers and they probably cross-react with each other. The n-ethylene glycol diacrylates and methacrylates tested are weak or non-sensitizers. TPGDA is a moderate and NPGDA a strong sensitizer. They were however, not pure. Neopentyl glycol dimethacrylate (NPGDMA) is a non-sensitizer. The commercial PETA is a mixture of pentaerythritol tri- and tetraacrylate (PETA-3 and PETA-4). PETA-3 is a much stronger sensitizer than PETA-4. Simultaneous reactions were seen between PETA-3, PETA-4 and TMPTA. TMPTA is a strong sensitizer and cross-reacts with PETA, but not with TMPTMA. The oligotriacrylate OTA 480 is a moderate sensitizer, but no concomitant reactions were seen with PETA-3, PETA-4 and TMPTA. Of the multifunctional acrylates tested, the di- and triacrylic compounds should be regarded as potent sensitizers. The methacrylated multifunctional acrylic compounds are weak or non-sensitizers.

The monofunctional acrylate (VI) 2-HPMA, is a weak sensitizer. The data suggest cross-reactivity or concomitant reactivity to 2-HEMA.

The influence of the test vehicle (IX) on the test response was also investigated. A great discrepancy between the test results for various acrylic compounds, is seen when petrolatum and acetone are used as test vehicle. The number of positive animals, as well as the mean response figures, are markedly decreased, when the various acrylates were tested in acetone. By using NPGDA as an acrylic monomer model, the same decrease in the test response was seen not only with acetone, but

with alcohol as well, as vehicle compared to petrolatum. By changing the test site location, half of the animals did not react when challenge test were applied near the abdomen, compared to a patch site close to the back, with petrolatum as vehicle.

The amount of acrylic monomer (NPGDA) in the test solutions, was found to decrease with increasing time. There was also a marked difference in the monomer residue between solutions with and without inhibitor. No adsorption of the acrylic monomers to the aluminium surface or filterpaper disc occurs. The discrepancy between the test results, when petrolatum and acetone were used as test vehicles is most certainly due to a polymerization process of the acrylic compounds. The petrolatum vehicle probably prevents the acrylic monomer from polymerization.

The sensitizing potential of the acrylates, was not influenced by the inhibitor. No animals reacted positively when tested with the inhibitor.

The purity of all acrylic compounds tested was analysed by HPLC. The di(meth)-acrylates based on bisphenol A or epoxy resin, were purified, and the main fractions were isolated and collected for further GPM-tests. The main fractions were analysed on NMR.

As many of the acrylic compounds tested were of commercial grade, sensitization to impurities cannot always be excluded. This emphasizes the importance of a pure substance, when discussing allergenicity and cross-reaction.

It is very important to perform predictive animal tests for allergenicity and to do careful chemical investigations of a product, before it is introduced on the market. Knowledge about its sensitizing capacity, its chemistry and contaminants is basic when discussing preventive measures.

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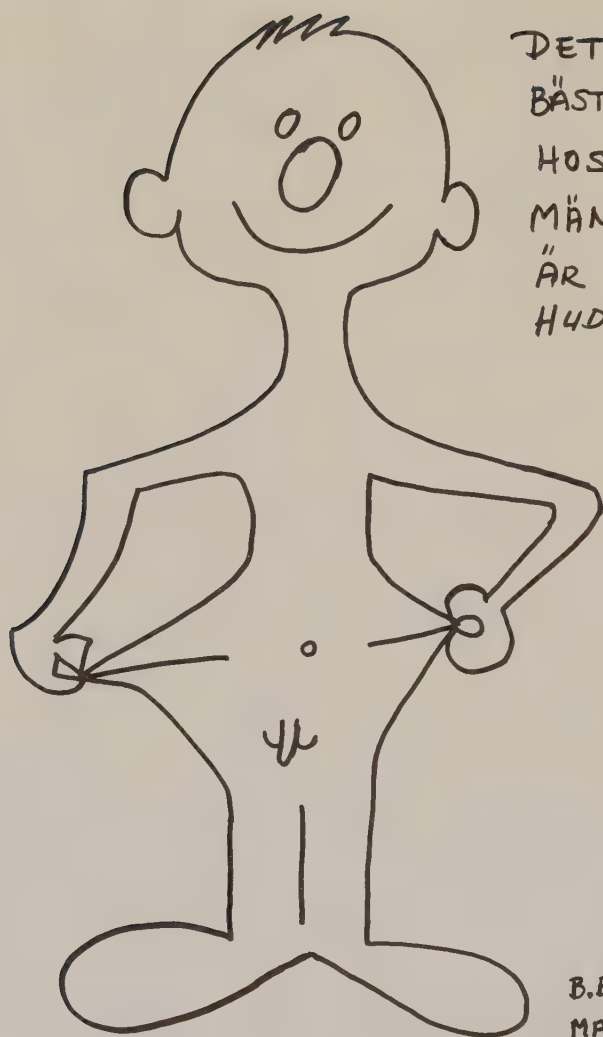
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I

ALLERGIC CONTACT DERMATITIS FROM
ACRYLATES IN ULTRAVIOLET CURING INKS

Bert Björkner, Inger Dahlquist and Sigfrid Fregert

Allergic contact dermatitis from acrylates in ultraviolet curing inks

BERT BJÖRKNER, INGER DAHLQUIST AND SIGFRID FREGERT

Department of Occupational Dermatology, University Hospital, Lund

Department of Dermatology, General Hospital, Malmö, Sweden

Six patients developed dermatitis while working with ultraviolet curing inks in four different printing plants. Contact allergy was found to the multifunctional acrylate monomers pentaerythritol triacrylate (PETA) and trimethylol propane triacrylate (TMPTA) and to the epoxy acrylate and polyesteracrylate prepolymers.

Key words: Bisphenol A dimethacrylate – contact allergy – curing ink – epoxy acrylate – pentaerythritol triacrylate – polyesteracrylate – trimethylol propane – trimethylol propane trimethacrylate – ultraviolet.

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During the last year we have seen six patients with dermatitis while working with ultraviolet curing inks in four printing plants in southern Sweden.

Skin problems associated with acrylates in ultraviolet curing inks or varnishes have been reported in the literature in recent years (Nethercott 1977, Smith 1977, Emmett 1977, Emmett & Kominsky 1977, Malten 1979, Malten et al. 1979). Several advantages of ultraviolet curing system compared to conventional printing technology have made many printing companies invest in the new method. More cases of dermatitis can be expected and six recent cases are reported here.

Case Reports

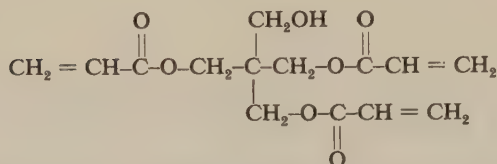
Out of six patients, one (Case 6) had atopic dermatitis as a child. The symptoms started in three patients (Cases 1, 4 and 5) 3–4 weeks after they had begun working with ultraviolet curing inks. Two patients had worked for 6 months (Cases 2 and 3) and one (Case 6) for 8 months. The six patients

worked at four different printing plants where the printing method had recently been introduced. At two plants the patients handled the printing press alone (Cases 2, 5 and 6) and one of these (Case 6) took over the work from Case 5 when he acquired eczema. At the other plants, where they had shift work, three of the six men working with the inks became sensitized.

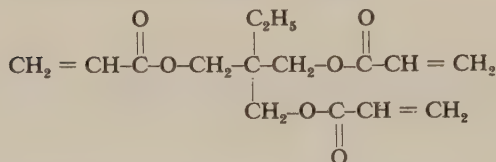
Symptoms usually started with itching of the forearms and face and eye irritation. One to 2 weeks later they developed dermatitis with erythema and scaling. Those who continued to work (Cases 1 and 2) developed severe eczematous reactions after several further weeks. Five out of six patients had dermatitis on the face (Cases 1, 2, 3, 4 and 5), three on the forearms (Cases 1, 2 and 4) and three on the hands (Cases 2, 3 and 6).

Patch tests

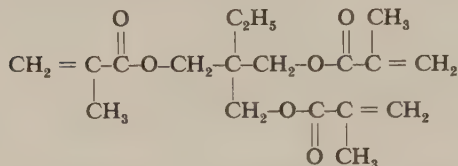
All patients were patch tested with the standard ICDRG series of contact allergens and the ink with which they had been in



Pentaerythritol triacrylate (PETA)



Trimethylol propane triacrylate (TMPTA)



Trimethylol propane trimethacrylate (TMPTMA)

Fig. 1. Structural formulas of multifunctional acrylates tested.

contact. Tests were done after the patients had healed completely. Patch tests were performed later with the components of the ink. The multifunctional acrylate in all inks was trimethylol propane triacrylate (TMPTA) (Fig. 1) and the prepolymer was either a polyesteracrylate (Fig. 2) or a diacrylate ester of bisphenol A epoxy resin (called an epoxy acrylate) (Fig. 3).

Patch tests were also performed with two other multifunctional acrylates reported to be used in this type of printing ink: pentaerythritol triacrylate (PETA) and trimethylol propane trimethacrylate (TMPTMA) (Fig. 1). TMPTMA has a molecular structure similar to that of TMPTA (Fig. 1).

Also patch tested was another prepolymer chemically related to epoxy acrylate, a di-

functional unsaturated methacrylic monomer based on bisphenol A (called bisphenol A dimethacrylate) (Fig. 3). It is said to cause less irritation and be less allergenic. None of the patients had been exposed to bisphenol A dimethacrylate before.

Four of the patients were also patch tested with the acrylic monomers, methyl methacrylate, ethyl acrylate, butyl acrylate and 2-ethyl-hexyl acrylate. Thirty control subjects were also tested with the ink and the same chemicals as the patients.

Results

Results of patch test with acrylates are summarized in Table 1. Case 1 also reacted to chromate, cobalt and wood tars and

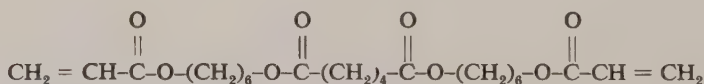
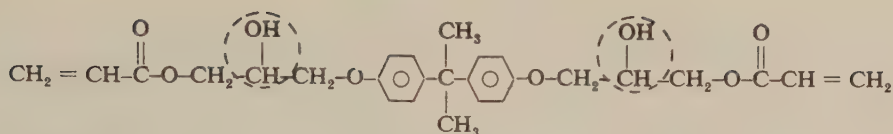


Fig. 2. Structural formula of polyesteracrylate.



Acrylated epoxy resin

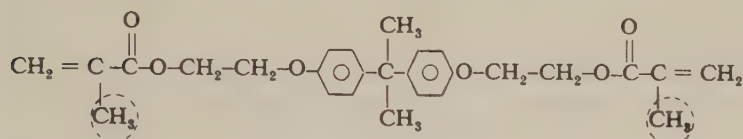


Fig. 3. Diacrylate ester of bisphenol A epoxy resin (epoxy acrylate) and methacrylic monomer based on bisphenol A (bisphenol A dimethacrylate).

Case 5 to neomycin. All patients reacted to the ink to which they had been exposed.

Reactions to ink constituents

All patients reacted to TMPTA and two had very strong reactions with erythema, spreading papules and coalescing vesicles. Of the prepolymers tested, three patients reacted to polyesteracrylate (Cases 1, 2 and 4) and two to epoxy acrylate (Cases 1 and 2).

Reactions to related compounds not present in the inks

Four patients out of six were positive to

PETA (Cases 1, 2, 3 and 4) but none reacted to TMPTMA. There were no reactions to bisphenol A dimethacrylate.

Of the four patients tested with acrylic monomers, none reacted to methyl methacrylate, one to ethyl acrylate (Case 1), two to 2-ethyl-hexyl acrylate and butyl acrylate (Cases 1 and 2).

None of the 30 control subjects tested reacted to the ink but four showed slight outlined brown-red erythema without infiltration with PETA, TMPTA and polyesteracrylate. These reactions were considered to be of weak irritant type. None of the

Table 1. Results of patch testing with acrylates in six men with dermatitis from UV curing inks

Case no.	1	2	3	4	5	6
Acrylate						
TMPTA* 0.5 % ac (v/v)	+++	+++	N.T.	++	++	N.T.
0.1 % ac (v/v)	N.T.	N.T.	++	+	N.T.	+
PETA 0.1 % ac (v/v)	+	+	++	+	—	—
TMPTA 1 % pet (v/w)	—	—	—	—	—	—
Prepolymers						
Used ink						
2 % pet (v/w)	++	++	++	++	++	+
Polyesteracrylate*						
1 % ac (v/v)	+	++	—	+	—	—
Epoxy acrylate*						
2 % ac (v/v)	++	+	—	—	—	—
0.5 % ac (v/v)	—	+	—	—	—	—
Bisphenol A dimethacrylate						
1 % ac (v/v)	—	—	—	—	—	—

* present in inks used.

controls reacted to any of the other chemicals.

Discussion

All patients gave positive patch test reactions to the ink that they worked with and to TMPTA, the multifunctional acrylate present in the inks used. Three of the six patients reacted to polyesteracrylate and two to epoxy acrylate, both compounds present as prepolymers in the inks used. Tests with bisphenol A dimethacrylate were performed to investigate if any cross-reaction occurred, because the molecular structures of bisphenol A dimethacrylate and epoxy acrylate are related (Fig. 3). None of the patients reacted to bisphenol A dimethacrylate.

The four men positive to PETA had previously worked with other ultraviolet curing inks, but information about the presence of PETA in these inks was impossible to obtain from the manufacturer. Cross-reaction between PETA and TMPTA is a possibility (Björkner 1980a).

No patients reacted to TMPTMA. Guinea pig sensitization work confirms that there is no cross-reaction from TMPTA to TMPTMA (Björkner 1980b).

Why certain patients also reacted positively to some of the acrylic monomers is difficult to explain. According to the manufacturer these components were not present in the inks used. It is known, however, that these acrylic monomers sometimes are added to printing inks (Rybny et al. 1974).

All patients were found to be allergic to the ink used as all reacted with positive patch tests and control subjects were all negative. Four controls out of 30 had slight brown-red outlined erythema without infiltration with PETA, TMPTA and polyesteracrylate. These were considered to be weak irritant reactions. In the patients, tests with the same chemicals and concentrations

showed spreading papules and vesicles with erythema and infiltration. In the literature patch tests have also been performed with higher concentrations (Emmett 1977, Emmett & Kominsky 1977). The irritant effect on human skin of the multifunctional acrylates has previously been discussed (Nethercott 1978, Malten et al. 1979). Even a patch test concentration of 0.1 % might be too high. To be quite sure of the sensitizing capacity of the acrylates used, guinea pig maximization tests have been carried out (Björkner 1980b).

Multifunctional acrylates, used as "diluent" and cross-linking monomers, are more volatile than the viscous reactive base polymers (Rybny et al. 1974) and because of the high speed of the rollers on the printing presses the inks also form microdrops in the air (Smith 1977). This air contamination might explain symptoms of itching on the face and forearms and irritation of the eyes. Patients who reacted to the prepolymers also admitted that they did not use gloves when refilling inks and cleaning the printing press. They were often contaminated with inks and varnishes of their hands and working clothes. It should be the duty of the manufacturer of the inks to inform the user of their sensitizing potential and to recommend preventive measures. Disposable gloves should be used when refilling inks and cleaning the press. It is important to wash immediately if inks contaminate the skin. It is also important to prevent the ink from contaminating the air by efficient exhaust ventilation.

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- Address:
B. Björkner
Section of Occupational Dermatology
Department of Dermatology
General Hospital S - 214 01 Malmö
Sweden

II

ALLERGENICITY OF TRIMETHYLOL PROPANE

TRIACRYLATE IN ULTRAVIOLET CURING

INKS IN THE GUINEA PIG

Bert Björkner

Allergenicity of Trimethylol Propane Triacrylate in Ultraviolet Curing Inks in the Guinea Pig

Bert Björkner

Department of Occupational Dermatology University Hospital, Lund and Department of Dermatology General Hospital, Malmö, Sweden

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Abstract. Trimethylol propane triacrylate (TMPTA) is a multifunctional acrylate commonly used in ultraviolet (UV) curing inks. Six men developed dermatitis when working with such UV curing inks in printing plants and all had positive patch test to TMPTA. The sensitizing capacity of TMPTA as determined by the "Guinea pig maximization test" (GPM) showed it to be a strong allergen. Cross-reaction with penta-erythritol triacrylate (PETA) occurs, but not with trimethylol propane trimethacrylate (TMPTMA).

Key words: Sensitizing capacity; Ultraviolet curing inks; Cross-reaction; Trimethylol propane triacrylate; Penta-erythritol triacrylate; Trimethylol propane trimethacrylate

The resins used in UV-cured inks are acrylated prepolymers diluted with multifunctional acrylates. The multifunctional acrylates have a dual role in UV-curable coatings or inks. One is to serve as cross-linking sites for the reactive base prepolymer and the other is to function as diluents for the usually more viscous base resins (12). In general, acrylic prepolymers and monomers are relatively transparent and consequently unable to initiate a fast polymerization without a photoinitiator, such as benzophenone.

In recent years reports have appeared about dermatitis among those working with UV-curing inks at printing plants or among workers manufacturing UV-curing resins (3, 4, 9, 10, 11, 13).

The advanced technology of using UV-curing inks at printing plants, has recently been introduced in Sweden. We have seen 6 patients with dermatitis after working with UV-curing inks at printing plant in southern Sweden (1).

The manufacturers informed us that the most commonly used inks at the printing plants in question consisted of trimethylol propane triacrylate (TMPTA) and diacrylate ester of bisphenol A epoxy resin (called epoxy acrylate). The inks also contained a benzophenone as a photoinitiator.

According to the manufacturers the inks were "tested for allergy". Most commonly the tests performed were the skin irritation test of Draize (2). The "Guinea pig maximization test" (GPM) (6, 7) was performed with some inks as such but not with the constituents separately. The differences

between, and performance and assessing of the test methods and results have caused much confusion among the manufacturers and users.

The purpose of the present investigation was to assess, with the "Guinea pig maximization test", the sensitizing capacity of TMPTA and to confirm the clinical test results (1). It was investigated whether cross-reactions occur with other multifunctional acrylates having similar molecular structures: penta-erythritol triacrylate (PETA) and trimethylol propane trimethacrylate (TMPTMA) (Fig. 1). The animals were also tested with acrylic acid, corresponding to the end groups of the tested compounds.

MATERIAL AND METHODS

Induction and challenge was performed in accordance with the original description of the GPM test (5, 6, 7).

Animals. Albino female guinea pigs, weighing 300–400 grams, were used.

Chemicals. Induction was performed with TMPTA. Challenges were made with TMPTA, TMPTMA, PETA and acrylic acid. The chemicals used were commercial products supplied by the manufacturer and reported to be pure.

Topical irritancy. The topical irritancy of the chemicals was studied with a 24-hour occluded patch test in 6 other animals than those used in the main test. Challenge patch test concentrations were used which did not give any irritant reactions.

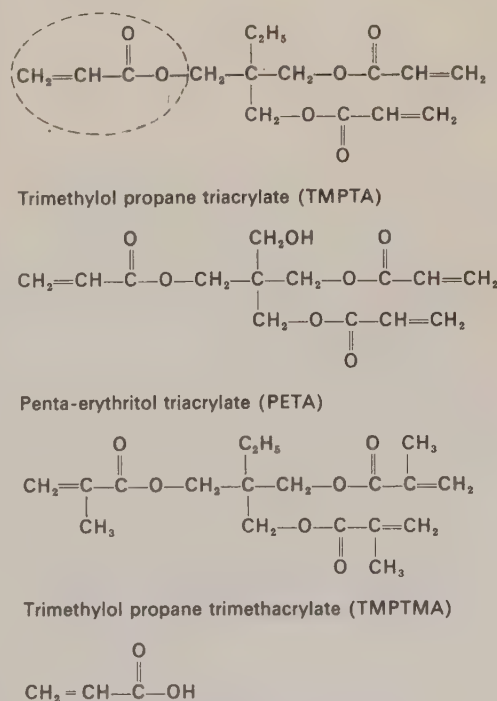


Fig. 1. Structured formulae of TMPTA, PETA, TMPTMA and acrylic acid.

Sensitization concentrations. Preliminary investigations were performed to determine the optimal sensitization concentration of TMPTA for intradermal induction in 6 animals without causing systemic toxicity.

Intradermal injections for induction of sensitivity. Three injections of the following mixtures were made in a row on each side of the shoulder region:

0.1 ml Freund's complete adjuvant (CFA) (Difco Lab., Detroit, Mich.) blended with an equal amount of water. 0.1 ml of TMPTA in an appropriate vehicle and concentration (Table I). 0.1 ml of a mixture containing the test substance in the vehicle and an equal amount of CFA.

Topical application for induction of sensitivity. One week after the injections the same shoulder area was clipped and shaved, after which closed patch exposure to the test substance in a vehicle was performed. The allergen in petrolatum was spread over a 2 by 4 cm patch of Whatman 3MM paper in a thick, even layer. The patch was covered by an overlapping, impermeable plastic adhesive tape (Leukoflex, Beiersdorf AG, Hamburg). This in turn was firmly secured by an elastic adhesive bandage (Acrylastic, Beiersdorf AG, Hamburg). The dressing was left in place for 48 hours. The final concentrations of the test substance for intradermal and topical inductions are given in Table I.

Table I. Concentrations of acrylates for sensitization and challenge

Acrylate	Sensitization		Challenge (%W/V)
	Intradermal (%W/V)	Topical (%W/V)	
TMPTA	1.0 in olive oil	25.0 in petrol	0.5 and 0.1 in petrol
PETA			0.5 and 0.1 in petrol
TMPTMA			0.5 in petrol
Acrylic acid			0.5 in petrol.

Challenge. Two weeks after the second stage of sensitization a 24-hour occluded patch test (Finn-chamber on Scanpor, Norgesplaster A/S, Norway, firmly secured by Acrylastic, Beiersdorf, AG, Hamburg) was performed on the flank after the area had been clipped and shaved. The animals were tested with TMPTA and PETA, both in a concentration of 0.5% and 0.1% in petrolatum (Table I). Three hours prior to the reading, the test site was again shaved with electric razor. Only evident redness and/or swelling was regarded as an allergic response.

Rechallenge. One week after challenge the animals were rechallenged with TMPTA, TMPTMA and acrylic acid, all in a concentration of 0.5% in petrolatum (Table I).

Controls. At the same time as the animals in the experimental groups were sensitized, the control animals in each series were also exposed intradermally to CFA and vehicle, and topically to the vehicle. When the sensitized animals in each series were challenged, control animals were also patch tested with the same acrylates in the same concentrations.

RESULTS

The test results are summarized in Table II. Sixteen (67%) of the 24 animals exposed to TMPTA be-

came sensitized. Even when the test concentration was as low as 0.1%, 6 of the 24 animals (25%) reacted positively. Eighteen of 24 animals (75%) also reacted to PETA in a 0.5% test concentration and 12 (50%) to 0.1%. None of the control animals,

Table II. Challenge reactions in 24 animals sensitized to TMPTA

Challenge concentrations in petrolatum

Animals	TMPTA		PETA	
	0.5%	0.1%	0.5%	0.1%
Number	16	6	18	12
Per cent	67	25	75	50
Controls	0	0	0	0

Table III. Cross-reactions in 24 animals sensitized to TMPTA

Challenge concentrations in petrolatum

Animals	TMPTA 0.5%	TMPTMA 0.5%	Acrylic acid 0.5%
Number	7	0	0
Per cent	29	0	0
Controls	10	0	0

tested simultaneously, reacted to any of the compounds.

When the animals were rechallenged one week later, 7 of 24 (29%) reacted to TMPTA, but none to TMPTMA and acrylic acid. However, 10 of the 24 control animals (40%) now reacted positively to TMPTA, but none to the other two compounds (Table III).

DISCUSSION

Clinical experience shows that workers exposed to UV-curing inks at printing plants can become sensitized. Presumably, many workers assume the inks are harmless and therefore fail to take appropriate precautions, as manufacturers have given information that "allergy tests" have been made and have shown that the inks are not "allergenic".

67% of the guinea pigs were sensitized to TMPTA, which can therefore be classified as a strong sensitizer (6, 7).

Of the TMPTA-sensitized animals, 75% also reacted to PETA, suggesting cross-sensitivity between the two substances. PETA has been shown to be a strong allergen (11).

When rechallenged one week later, 40% of the control animals reacted positively to TMPTA. They had been sensitized by only one application of TMPTA at the challenge procedure one week earlier, which also shows the high rate of allergenicity

of TMPTA. No cross-reaction occurred between TMPTA and TMPTMA or acrylic acid (Table III).

The results indicate that the whole molecule of TMPTA—and not merely the acrylic parts—acts as an allergen (Fig. 1). It is rather difficult to sensitize guinea pigs to acrylic acid (8).

The methyl groups in the molecule of TMPTMA seem to influence the allergenicity. By using acrylates substituted with methyl groups the allergenic potential might be diminished and could be one way of making less hazardous compounds.

It should be stressed that it is important to perform the guinea pig maximization test of the ingredients in new products before marketing. In view of the results of the present investigation, proper prevention measures should be performed.

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III

SENSITIZATION CAPACITY OF ACRYLATED PREPOLYMERS IN ULTRAVIOLET CURING INKS TESTED IN THE GUINEA PIG

Bert Björkner

SENSITIZATION CAPACITY OF ACRYLATED PREPOLYMERS IN ULTRAVIOLET CURING INKS TESTED IN THE GUINEA PIG

Bert Björkner

*Department of Occupational Dermatology, University Hospital, Lund, and Department of Dermatology,
General Hospital, Malmö, Sweden*

Abstract. One commonly used prepolymer in ultraviolet (UV) curing inks is epoxy acrylate. Of 6 men with dermatitis contracted from UV-curing inks, 2 had positive patch test reaction to epoxy acrylate. None reacted to the chemically related bisphenol A dimethacrylate. The sensitization capacity of epoxy acrylate and bisphenol A dimethacrylate performed with the "Guinea pig maximization test" (GPM) shows epoxy acrylate to be an extreme sensitizer and bisphenol A dimethacrylate a moderate sensitizer. Cross-reaction between the two substances occurs. The epoxy resin oligomer MW 340 present in the epoxy acrylate also sensitized some animals.

Key words: Acrylic acid; Acrylated prepolymer; Bisphenol A dimethacrylate; Cross-reaction; Epoxy acrylate; Epoxy resin; Methylmethacrylate; Sensitization capacity; Ultraviolet curing ink

The introduction of the ultraviolet (UV) curing inks in printing plants seems to have many advantages compared with conventional printing technology, such as reduction in energy consumption as well as elimination of air pollution (15). During the last year, however, reports have been published concerning skin problems experienced by those men working with or manufacturing UV-curing inks or varnishes (2, 4, 5, 12, 13, 14, 16).

UV-curing inks contain three basic components: a reactive base prepolymer, a photo-initiator (usually a benzophenone), and a multifunctional acrylic monomer used as a "diluent" with "cross-linking" properties.

The prepolymers are synthetic resins with terminal acrylate groups. The most common are acrylated polyesters, acrylated polyethers, acrylated urethanes and acrylated epoxy resins. In many formulations the base prepolymer portion of the inks constitutes as much as 80%.

In a previous investigation 6 men with dermatitis contracted from UV-curing inks, 2 had a positive

patch test reaction to the prepolymer used (2). According to the manufacturer this prepolymer was a diacrylate ester of bisphenol A epoxy resin (Fig. 1).

Some of the same features that give epoxy resins their superior performance in thermal systems are not always found with acrylated epoxy resins. They have too high a viscosity and impart excessive hardness to the coating. Other types of acrylated prepolymers have therefore been developed. One of these, a difunctional unsaturated methacrylic monomer based on bisphenol A (called bisphenol A dimethacrylate) is said to have such good properties that it can replace epoxy acrylates not only in UV-curing inks, but also in conventional epoxy resins (Fig. 1). Bisphenol A dimethacrylate is also believed by the manufacturer to be less irritating and allergenic and thus meets the requirements of safer handling and application.

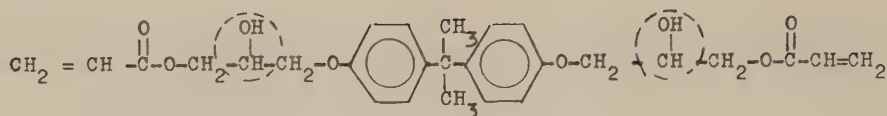
The purpose of this study was to assess with the "Guinea pig maximization test" (9, 10) the sensitizing capacity of epoxy acrylate and bisphenol A dimethacrylate and to investigate if any cross-reaction between these two chemically related prepolymers occurs and to confirm the clinical test results.

MATERIAL AND METHODS

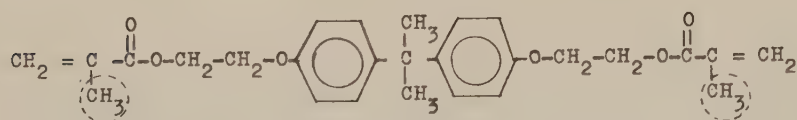
The induction and challenge were in accordance with the original description of the GPM test (7, 9, 10). A booster dose was also given intradermally with the sensitizing chemicals 48 hours after the challenge application.

Animals. Albino female guinea pigs, weighing 300-400 g, were used.

Chemicals. The acrylates used for sensitization and challenge were epoxy acrylate and bisphenol A dimethacrylate (Fig. 1). Challenge was also performed with epoxy resin of bisphenol A type (epoxy oligomer MW 340), bisphenol A, acrylic acid and methylmethacrylate (MMA). Thin-layer chromatography (6) revealed the presence of epoxy resin oligomer MW 340 in the epoxy acrylate but not in the bisphenol A dimethacrylate.



Acrylated epoxy resin



Methacrylic monomer based on bisphenol A

Fig. 1. Diacrylate ester of bisphenol A epoxy resin (epoxy acrylate) and methacrylic monomer based on bisphenol A (bisphenol A dimethacrylate).

Topical irritancy. The topical irritancy of the chemicals was studied by a 24-hour closed patch test in 6 animals not used in the test. A challenge patch test concentration was used which did not give any reaction.

Sensitization concentrations. Preliminary investigations were performed in 6 animals to establish the optimal sensitization concentration of the test substances for intradermal and topical induction without causing systemic toxicity.

The final concentrations chosen for intradermal induction with epoxy acrylate were 5% in liquid paraffin and with bisphenol A dimethacrylate 10% in liquid paraffin. For topical induction, both acrylates were used in 100%.

Challenge. All animals were tested with epoxy acrylate in a concentration of 10% in acetone and with bisphenol A dimethacrylate in a concentration of 25% in acetone. Only evident redness and/or swelling was regarded as an allergic response. The reactions were evaluated blind and an assistant chose the cages of the animals at random.

Cross-testing. In order to maintain the allergenic potential, those animals sensitized to epoxy acrylate received, 48 hours after challenge application, a booster dose with 0.1 ml of epoxy acrylate in a concentration of 5% in liquid

paraffin intradermally in the shoulder region (11). The control animals got the same amount of the vehicle alone and in the same way as the experimental group. One week later the animals were rechallenged with 10% epoxy acrylate, 1% epoxy oligomer MW 340, 1% bisphenol A, 0.5% acrylic acid and 1% methylmethacrylate, all in acetone.

The other group which was sensitized to bisphenol A dimethacrylate also got a booster dose intradermally above the shoulder region 48 hours after challenge application but with 0.1 ml of bisphenol A dimethacrylate 10% in liquid paraffin. In the same way, the control group received 0.1 ml of the vehicle alone. One week later the animals were rechallenged with 25% bisphenol A dimethacrylate, 1% epoxy oligomer MW 340, 1% bisphenol A, 0.5% acrylic acid and 1% methylmethacrylate, all in acetone.

Controls. At the same time as the animals in the experimental groups were sensitized, the control animals in each series were also exposed intradermally to Freund's complete adjuvant (CFA) and vehicle. When the sensitized animals in each series were challenged, control animals were also patch tested with the same chemicals and in the same concentrations.

Table I. Challenge reactions in 20 animals sensitized to epoxy acrylate

Challenge concentrations in acetone

Animals	Epoxy acrylate 10%	Bisphenol A dimethacrylate 25%
Number	18	9
Per cent	90	45
Controls	0	0

Table III. Challenge reactions in 20 animals sensitized to bisphenol A dimethacrylate

Challenge concentrations in acetone

Animals	Bisphenol A dimethacrylate 25%	Epoxy acrylate 10%
Number	8	14
Per cent	40	70
Controls	0	0

Table II. *Cross reactions in 20 animals sensitized to epoxy acrylate after a booster dose*
Challenge concentrations in acetone

Animals	Epoxy acrylate 10%	Epoxy MW 340 1%	Bis-phenol A 1%	Acrylic acid 0.5%	Methyl-methacrylate 1%
Number	20	5	1	1	0
Per cent	100	25	5	5	0
Controls	0	0	0	0	0

RESULTS

Induction with epoxy acrylate. The test results are summarized in Table I. Eighteen (90%) of 20 animals exposed to epoxy acrylate became sensitized. Nine animals (45%) also reacted to bisphenol A dimethacrylate. None of the control animals reacted.

The rechallenge of the animals one week after a booster dose with epoxy acrylate shows that 100% of the 20 animals were sensitized. Five (25%) of the animals reacted to epoxy oligomer MW 340, one to the bisphenol A, one to acrylic acid, but none to methylmethacrylate (Table II).

None of the control animals reacted to any of the compounds.

Induction with bisphenol A dimethacrylate. The test results are summarized in Table III. Eight (40%) of 20 animals exposed to bisphenol A dimethacrylate became sensitized. Fourteen animals (70%) also reacted to epoxy acrylate. None of the control animals tested simultaneously reacted.

After a booster dose with bisphenol A dimethacrylate, the rechallenge one week later showed that 9 (45%) of 20 animals were sensitized. Six of the 20 (30%) reacted positively to epoxy oligomer MW 340, 2 to acrylic acid but none to bisphenol A and methylmethacrylate. One of the 6 animals positive to epoxy oligomer 340 had a negative test reaction to bisphenol A dimethacrylate. None of the control

animals reacted to any of the compounds (Table IV).

DISCUSSION

Ninety per cent of the guinea pigs were sensitized to epoxy acrylate which can be classified as an extreme sensitizer (10). Forty-five per cent of the animals reacted positively when tested with bisphenol A dimethacrylate, suggesting a certain cross-reaction between epoxy acrylate and bisphenol A dimethacrylate. This is not in agreement with the previous clinical findings in which 2 of 6 men with dermatitis from UV-curing inks reacted to epoxy acrylate but none cross-reacted to bisphenol A dimethacrylate (2). However, this material is small. After a booster dose with epoxy acrylate and rechallenge, all animals became sensitized, a further indication of the strong sensitizing capacity.

Forty per cent of the guinea pigs were sensitized to bisphenol A dimethacrylate which can be classified as a moderate sensitizer (10), 70% of the animals also reacted to epoxy acrylate. It is surprising that the secondary allergen elicited reaction in more animals than did the primary allergen.

The only difference in molecule structure between epoxy acrylate and bisphenol A dimethacrylate is two hydroxyl- and methyl groups (Fig. 1). Epoxy acrylate is an extremely potent sensitizer compared with the moderate sensitizer bisphenol A

Table IV. *Cross reactions in 20 animals sensitized to bisphenol A dimethacrylate after a booster dose*
Challenge concentrations in acetone

Animals	Bisphenol A dimethacrylate 25%	Epoxy MW 340 1%	Bis-phenol A 1%	Acrylic acid 0.5%	Methyl-methacrylate 1%
Number	9	6	0	2	0
Per cent	45	30	0	10	0
Controls	0	0	0	0	0

dimethacrylate. Methacrylates are less potent sensitizers than corresponding acrylates (1).

Of the 20 animals sensitized to bisphenol A dimethacrylate, 6 also reacted to epoxy oligomer MW 340. TLC showed no presence of epoxy oligomer MW 340 (12). The reaction cannot be explained at present but requires further investigations.

One of the animals sensitized to bisphenol A methacrylate also reacted to bisphenol A and acrylic acid, which could be due to a hyperreactivity in this animal. It seems rather difficult to sensitize guinea pigs to acrylic acid (8).

Information from the manufacturer about tests made according to Draize for skin irritation (3) show that "the primary irritation index" for epoxy acrylate is less than 0.5 and non-irritant, and for bisphenol A dimethacrylate 1.7, which means slightly irritant. Compared with the sensitization class for these acrylates, it shows the discrepancy between skin irritation and the sensitizing capacity and it is important to inform manufacturers and workers about the difference between these two methods and between allergenic and irritant effects on the skin.

The epoxy acrylate molecule is formed by letting the epoxy oligomer react with acrylic acid, to impart acrylic-type terminal unsaturation to the prepolymer. It seems probable that the whole molecule of epoxy acrylate acts as an allergen and not the terminal acrylic groups, as they did not react to acrylic acid.

Of the animals sensitized to epoxy acrylate, 25% reacted positively to epoxy oligomer MW 340. TLC showed presence of free epoxy resin in the epoxy acrylate. The patients were not sensitized to epoxy resin. The guinea pig maximization test shows, however, that there is a potential risk of sensitization with the epoxy resin contaminant when working with epoxy acrylates in UV-curing inks.

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B. Björkner, M.D.
Section of Occupational Dermatology
Department of Dermatology
General Hospital
S-21401 Malmö
Sweden

IV

SENSITIZATION CAPACITY OF POLYESTER
METHACRYLATE IN ULTRAVIOLET CURING
INKS TESTED IN THE GUINEA PIG

Bert Björkner

SHORT REPORTS

Sensitization Capacity of Polyester Methacrylate in Ultraviolet Curing Inks Tested in the Guinea Pig

Bert Björkner

Department of Occupational Dermatology, University Hospital, Lund, and Department of Experimental Research, General Hospital, Malmö, Sweden

Received June 12, 1981

Abstract. One of the prepolymers used in Ultraviolet (UV) curing ink formulations is acrylated polyester. The prepolymer investigated is a dimethacrylated polyester, thus a saturated polyester terminated with methacrylic groups. In conventional UV-curing coatings the prepolymer commonly used is an unsaturated polyester. Three of six workers with dermatitis from UV-curing inks in printing plants had positive test results from the polyester methacrylate present in the inks used. The sensitizing capacity, performed with the "Guinea pig maximization test", shows polyester methacrylate to be a moderate sensitizer.

Key words: Acrylated prepolymers; Methylacrylate; Methyl methacrylate; Polyester methacrylate; Sensitization capacity; Ultraviolet curing ink; Unsaturated polyester

Ultraviolet light-cured inks have been formulated and used successfully in letterpress, lithographic, gravure and flexographic printing as a substitute for the solvent-based printing inks currently used. These new ultraviolet light (UV)-cured inks offer advantages other than the elimination of solvent emission, notably: a combination of excellent film properties with fast printing speeds; curing at ambient temperature with less damage to heat-sensitive substrates such as plastics, textiles, wood and paper; and a reduced energy consumption. The great interest in their development is shown by the large number of patient citations describing various compositions that can be cured by ultraviolet light (9, 11).

The unsaturated polymers used in the formulation of UV-curable resins can be divided into two

broad classes: unsaturated polyester resins and polymers containing pendent unsaturation.

In unsaturated polyester resins, the monomer or "solvent", primarily styrene or vinyl toluene, copolymerizes by fumarate or maleate double bonds which are randomly distributed in the polyester backbone. Peroxide-cured unsaturated polyesters have been used commercially for many years (5). According to the patent literature (11), UV-curable unsaturated polyesters are quite similar in composition to resins used commercially for peroxide cures. Properly UV-cured unsaturated polyesters have been found to give properties equivalent to peroxide-cured systems. The UV-method therefore has found its first application in the cure of these materials. The process is now used in furniture and cabinet manufacture where unsaturated polyesters find use as board fillers, top coats for furniture, doors, panels, panelling etc. (8, 9, 11).

During recent years orthopaedic casts cured by ultraviolet light have been increasingly used. Usually they consist of an unsaturated polyester with vinyl toluene as monomer and a benzoin-ether molecule as photo-initiator. The resin is impregnated into a fiberglass weave.

Suspicion regarding health hazards from inhalation of the monomers, styrene and vinyl toluene, is one of the reasons for developing the second major class of UV-curable polymers consisting of thermoplastic resins into which the reactive acrylate or methacrylate have been introduced.

The reactive acrylate groups are attached to the backbone of the resins through functional groups.

The prepolymers used in UV-curing ink formulations usually are synthetic resins with terminal acrylate groups. The most common are diacrylate esters of polyesters, polyethers, urethanes and epoxy resins. The commercially used UV-curing inks are a mixture of acrylated prepolymer, multifunctional acrylic monomer, photo-initiator and inhibitor.

During recent years, contact allergy has been reported from acrylates used in UV-curing inks (1, 2, 6, 7, 10, 12) and some have been proved to be potent sensitizers (3, 4, 14).

Of the 6 men with dermatitis, when working with

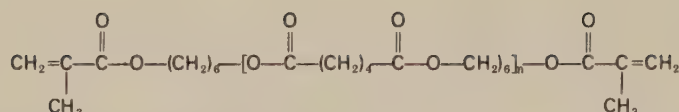


Fig. 1. Methacrylic oligomers based on polyester (methacrylated polyester).

UV-curing inks, discussed in previous investigations (1, 2), 3 reacted to the polyester methacrylate they had been exposed to.

The purpose of this study was to investigate the sensitizing capacity of the polyester methacrylate present in the ink used, with the "Guinea pig maximization test" (GPM test).

MATERIALS AND METHODS

The methods were principally the same as in previous work (3, 4) and were in accordance with the original description of the GPM test.

Animals. Albino female guinea pigs, weighing 300–400 g, were used. The animals were kept in the pen for a week before the experiments.

Chemicals. The polyester methacrylate used for sensitization and challenge was commercial available (Ebecryl 810, UCB, furnished by Grafisk Färg AB, Stockholm, Sweden) and used in the printing industry (Fig. 1).

Challenge was also performed with methyl methacrylate (MMA) and methylacrylate (MA).

Topical irritancy. The topical irritancy of the chemicals was studied by a 24-hour closed patch test in 6 animals not used in the test. A challenge patch test concentration was used which did not give any reaction.

Sensitization concentrations. Preliminary investigations were performed in 6 animals to establish the optimal sensitization concentration of polyester methacrylate for intradermal and topical induction without causing systemic toxicity.

The final concentration for intradermal induction with polyester-acrylate was 10% in liquid paraffin. For topical induction, 100% concentration was used. Pretreatment with sodium lauryl sulphate gave a too strong reaction.

Challenge. All animals were tested with polyester methacrylate in a concentration of 5% in acetone. Only evident redness and/or swelling was regarded as an allergic response. The reactions were evaluated blind and an assistant chose the cages of the animals at random.

Simultaneously with the challenge with the polyester methacrylate, the animals were also patch tested with MMA and MA, both in a concentration of 2% in acetone.

Controls. At the same time as the animals in the experimental group were sensitized, the control animals were also exposed intradermally to Freund's complete adjuvant (CFA) and vehicle. For the topical induction a dry cotton webril was used. When the sensitized animals were challenged the control animals were also patch tested with the same chemicals and in the same concentrations.

RESULTS

Six (33%) of 20 animals exposed to polyester methacrylate became sensitized. Methyl methacrylate and methylacrylate gave no reactions in the sensitized animals. There were no reactions at all in the control animals to any of the compounds.

The test results are summarized in Table I.

DISCUSSION

Thirty-three per cent of the guinea pigs were sensitized to polyester methacrylate, which can be classified as a moderate sensitizer.

Fifty per cent of the workers with dermatitis from UV-curing inks in the printing plant earlier discussed (1, 2) had positive test results from polyester methacrylate. The workers had been exposed fairly frequently to polyester methacrylate, a common prepolymer in the inks used.

The polyester methacrylate tested (Ebecryl 810, UCB, Belgium) is, according to the manufacturer, a dimethacrylated polyester, thus a saturated polyester terminated with methacrylic groups.

The unsaturated polyester prepolymer used in conventional UV-curing coatings is typically prepared from maleic anhydride, an aromatic anhydride and a diol and has many double bonds in its molecular structure, which can easily undergo radical changes important in the polymer formation (9).

To my knowledge, no allergic contact dermatitis

Table I. Challenge reactions in 20 animals sensitized to polyester methacrylate. Challenge concentrations in acetone

Animals	Polyester methacrylate 5%	Methyl methacrylate 2%	Methylacrylate 2%
Number	6	0	0
Per cent	33	0	0
Controls	0	0	0

caused by unsaturated polyesters has been reported.

Probably, the whole molecular structure of polyester methacrylate acts as an allergen, since none of the animals reacted when tested with methyl methacrylate or methacrylate. However, the reactive terminal methacrylate groups seem to be of great importance for antigen formation and sensitization.

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CORRECTIONS

The information given from the manufacturer about the chemical structure of Ebecryl 810 has been proven to be false. Ebecryl 810 is not a dimethacrylated polyester, but a polyester acrylate with 4 functional acrylate groups with a theoretical molecular weight of 750. The main component is a reaction product between a dicarboxylic acid and 2 molecules of a triol, which then is acrylated. Further informations are confidential. The test results are not influenced by this.

V

THE SENSITIZING POTENTIAL OF DI(METH)ACRYLATES

BASED ON BISPHENOL A OR EPOXY RESIN

IN THE GUINEA PIG

Bert Björkner, Bo Niklasson and Karin Persson

THE SENSITIZING POTENTIAL OF DI(METH)ACRYLATES BASED ON BISPHENOL A OR EPOXY RESIN IN THE GUINEA PIG

Bert Björkner, Bo Niklasson and Karin Persson

Section of Occupational Dermatology, Department of Dermatology, General Hospital, Malmö, Department of Occupational Dermatology, University Hospital, Lund and Department of Experimental Research, General Hospital, Malmö, Sweden. Contact Dermatitis (Accepted for publication)

Most composite materials in dentistry used today, contain resins based on dimethacrylates. BIS-GMA [2,2-bis(4-(2-hydroxy-3-methacryloxypropoxy)phenyl)propane], the addition reaction product of bisphenol A and glycidyl methacrylate or an epoxy resin and methacrylic acid, is used most extensively. More recently, dimethacrylates based on bisphenol A, with various chain lengths have appeared on the market as a substitute for or in addition to BIS-GMA. Such compounds are BIS-MA [2,2-bis(4-(methacryloxy)phenyl)propane], BIS-EMA [2,2-bis(4-(2-methacryloxyethoxy)phenyl)propane] and BIS-PMA [2,2-bis(4-(3-methacryloxypropoxy)phenyl)propane]. Increasing interest in the radiation cure of coatings and printing inks have focused attention on these substances and on epoxy diacrylates as radiation-curable resins. The sensitizing capacity of the different acrylates based on bisphenol A or epoxy resin have been investigated with the "Guinea Pig Maximization Test". The pattern of simultaneous reactivity of the compounds was also studied. Epoxy diacrylate [2,2-bis(4-(2-hydroxy-3-acryloxypropoxy)phenyl)propane]. BIS-EMA and BIS-MA are shown to be strong sensitizers, while the linear fraction of BIS-GMA and its isomers and BIS-PMA have none or a low sensitizing capacity. The impurities in the BIS-GMA and BIS-MA batches seem to have high allergenic potential. These results stress the importance of a pure substance when discussing allergenicity and cross reactions.

Key words: Bisphenol A - BIS-GMA - 2,2-bis[4-(2-hydroxy-3-methacryloxypropoxy)phenyl]propane - BIS-EMA - 2,2-bis[4-(2-methacryloxyethoxy)phenyl]propane - BIS-MA - 2,2-bis[4-(methacryloxy)phenyl]propane - BIS-PMA - 2,2-bis[4-(3-methacryloxypropoxy)phenyl]propane - cross-reaction - dental restorative material - epoxy acrylate - 2,2-bis[4-(2-hydroxy-3-acryloxypropoxy)phenyl]propane - epoxy resin - guinea pig maximization test - hydroquinone - sensitizing capacity - UV-curing acrylate.

Composite restorative materials have largely displaced silicate cements and unreinforced filling resins in dentistry. The term "composite" refers to a mixture of filler and a minor amount of resin binder. Most composite materials marketed now contain special resins based on dimethacrylates. Of the dimethacrylate monomers used as hardenable binders for the reinforcing fillers, BIS-GMA or epoxy dimethacrylate, [the addition reaction product of bisphenol A; 2,2-bis-(4-hydroxyphenyl)propane and glycidyl methacrylate or an epoxy resin and methacrylic acid] is now the most extensively used (1). BIS-GMA is short for the chemical name: 2,2-bis[4-(2-hydroxy-3-methacryloxypropoxy)phenyl]propane. The structural formula is shown in Fig. 1.

The synthesis of BIS-GMA had its beginnings in the early 1950's in attempts to use commercial epoxy resins with various hardening agents as a binder for reinforcing fillers. Epoxy resins were not suitable, however, because moisture interfered with its polymerization (1). This prompted an attempt to synthesize a hybrid monomer by letting epoxy resin react with acrylic or methacrylic acid. This was first described by Bowen (2) in the early 1960's.

Among producers, there have been problems associated with BIS-GMA used as dental resin; for example, its high water sorption and viscosity (3, 4). Several variants have appeared as substitutes for BIS-GMA or in addition to BIS-GMA. By letting the -OH group in the BIS-GMA structure react with acetic anhydride or other acetylating agents, the water sorption instability is reduced. Therefore, more recently, dimethacrylates based on bisphenol A, with various chain lengths, that do not contain a hydroxyl group have been synthesized. Such compounds are BIS-MA; 2,2-bis[4-(methacryloxy)phenyl]propane, BIS-EMA; 2,2-bis[4-(2-methacryloxyethoxy)phenyl]propane and BIS-PMA; 2,2-bis[4-(3-methacryloxypropoxy)phenyl]propane (Fig. 1). BIS-GMA is, however, the resin matrix in almost all commercially available composite restorative materials and fissure sealants used today.

Some also contain BIS-MA and BIS-EMA, while a few only consist of BIS-PMA. Many of the composite materials are diluted with low viscous acrylic and methacrylic monomers e.g. ethylene glycol dimethacrylate (EGDMA), diethylene glycol dimethacrylate (DEGDMA), triethylene glycol dimethacrylate (TREGDMA) and methyl methacrylate (MMA) (5, 6).

In addition to serving as the principal monomer in dental composite and

sealants, BIS-GMA resins and other similar derivatives are finding extensive use in industrial applications (7 - 13).

Increasing interest in the radiation cure of coatings or printing inks, by means of high-energy electrons or ultraviolet radiation, focused attention on these materials as radiation curable resins. The reaction product of an epoxy resin, e.g. based on bisphenol A diglycidyl ether, with a vinyl acid, such as acrylic or methacrylic acid, have been referred to as epoxy acrylates.

The properties of epoxy resins and acrylates combined, have a major influence on their utility in radiation-curable systems. The epoxy resin part provide the toughness, adhesion and chemical resistance, while the terminal acrylic functionality imparts high free-radical reactivity.

Epoxy acrylates have their most useful applications where moderate adhesion to a metal surface is required, and on wood, particle board and paper coatings and in printing inks where a highly reactive system is desired (14, 15).

Polymerization of acrylates based on bisphenol A or epoxy resin is either chemically activated by use of peroxides or activated by the exposure of an initiator to electron beam or ultraviolet light to generate the free radicals which initiate the final reactions.

Inhibitors are important ingredients in any formulation involving free radical chemistry. These retard or prevent polymerization until cure is desired. Among the most used inhibitors are those of phenolic type e.g. hydroquinone or its derivatives. Most unsaturated resins and monomer diluents contain small amounts, e.g. 15-1000 u/g of inhibitors.

Recently, contact allergic reactions have been reported to BIS-GMA and epoxy acrylates (16 - 20).

The purpose of this study was: (i) to investigate the sensitizing capacity of diacrylates based on bisphenol A or epoxy resin with "The Guinea Pig Maximization Test" (21, 22); (ii) to investigate the pattern of simultaneous reactivity between certain fractions of the compounds.

MATERIAL AND METHODS

Chemicals.

The following diacrylates based on bisphenol A or epoxy resin were used in the study.

1. Epoxy acrylate XD 8008.04 (DOW Chemical Europe S.A., Switzerland) here called Epoxy acrylate DOW, and Epicote acrylate DRH-370 (Shell Chemicals, The Netherlands) were investigated. Both acrylate esters are derived from a low molecular weight bisphenol A epoxy resin. The chemical name is 2,2-bis[4-(2-hydroxy-3-acryloxypropoxy)phenyl]propane (Fig. 1).

2. BIS-PMA, 2,2-bis[4-(3-methacryloxypropoxy)phenyl]propane (ESPE GmbH, West Germany) has a linear structure, i.e. the 1,3-propane dioxy derivative (Fig. 1).

3. BIS-EMA, 2,2-bis[4-(2-methacryloxyethoxy)phenyl]propane is the 1,3-ethane dioxy derivative (Fig. 1). A commercially available BIS-EMA, Diacryl 101, which is the trade name used by AKZO Chemie, The Netherlands, was used for induction. For investigation of cross reactivity, another brand of BIS-EMA from Ancomer Ltd, England was used.

4. BIS-GMA, 2,2-bis[4-(2-hydroxy-3-methacryloxypropoxy)phenyl]propane is a reaction product of bisphenol A and glycidyl methacrylate or a reaction product of glycidyl ether of bisphenol A and methacrylic acid (Fig. 1). The BIS-GMA used in this investigation was furnished by 3 M Company, Minnesota, USA.

5. BIS-MA, 2,2-bis[4-(methacryloxy)phenyl]propane, manufactured by Aldrich-Europe, Belgium, is the reaction product of bisphenol A and methacrylic acid (Fig. 1).

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY - ANALYSIS

Apparatus. An isocratic high performance liquid chromatography (HPLC) system was used (Laboratory Data Control, LDC, Milton Roy Company, USA), comprising a LDC Constametric III pump and a variable UV-detector, LDC Spectro Monitor III. A Perkin Elmer 550 S UV/VIS spectrophotometer was used for determination of absorption maximum, in order to choose the optimal wavelength. For the collection of fractions, a LKB 2112 Redirac fraction collector was used. Nuclear

Magnetic Resonance (NMR) was performed at the Chemical Center, University of Lund.

Columns. The following columns were used for analysis, separation and isolation.

1. Nucleosil C-18, 5 μ , (Macherey-Nagel & Co., Düren) 200 mm x 4.6 mm, i.d.
2. Sperisorb ODS, 5 μ , (Phase Separations Ltd, U.K.) 250 mm x 4.6 mm, i.d.
3. Nucleosil CN, 5 μ , (Macherey-Nagel & Co., Düren) 200 mm x 4.6 mm, i.d.
4. Partisil M 9, 10 μ , semipreparative, (Whatman, USA) 250 mm x 9.4 mm, i.d.
5. Partisil ODS-2, 10 μ , semipreparative, (Whatman, USA) 250 mm x 9.4 mm, i.d.
6. Merck RP-18, 10 μ , semipreparative, (E. Merck, Darmstadt, (GFD) slurry packed in a stainless steel column: 250 mm x 9.4 mm, i.d.

Procedure. The purity of the products was analysed by the HPLC-system with various columns and wavelengths (Table 1). For collection of fractions, semi-preparative columns were used (Table 2). The purity of the fractions was then controlled by reinjecting them into the analytical column (Table 1). To prevent polymerization of the BIS-MA fractions, thymol was added as inhibitor to the main fraction and thymol and hydroquinone were added to the pre-fraction. From the fractions, the solvent was finally evaporated and the residue stored in a refrigerator. NMR-analysis was performed on each main fraction to confirm the chemical structure.

The Guinea Pig Maximization Test (GPM-test)

The GPM-test was performed in accordance with the original description (21, 22).

Animals. For every substance tested, 30 female albino guinea pigs of the Hartley-Dunkin strain, weighing 300-400 g, were used, 15 in an experimental group and 15 in a control group.

Chemicals. Induction was performed with the commercial acrylic products described above and with the main fractions (Table 3). The following chemicals were also used for challenge: bisphenol A (Fluka AG, Switzerland), glycidyl methacrylate (Perstorp Chemie, Sweden) methacrylic acid (Perstorp Chemie, Sweden), acrylic acid (Perstorp Chemie, Sweden), epoxy resin MW 340 (Shell Chem., The Netherlands), hydroquinone (Fluka AG, Switzerland), triphenyl antimony (Fluka AG, Switzerland) and triphenylphosphine (Fluka AG, Switzerland).

Topical irritancy. The topical irritancy of the chemicals in serial dilutions was studied by a 24 h closed patch test in 6 animals not used in the test procedure. Challenge patch test concentrations were used which did not give any reactions.

Intradermal reaction and systemic toxicity. Preliminary investigations were performed in 6 animals with 3 different concentrations to establish the optimal sensitization concentration of the test substances for intradermal induction without causing systemic toxicity.

Induction procedure. The vehicle for intradermal injections was olive oil for most of the substances. Some of the test substances are rather viscous, and in order to get a better dispersion for intradermal injection, they were dissolved in a mixture of olive oil and acetone (9:1). The Freund's Complete Adjuvant (FCA) used was purchased from Difco Laboratories, Detroit, Mich., USA. 24 h prior to topical application of the test substances, the area was pretreated with 10% sodium lauryl sulphate (SLS) in pet. without occlusion. The SLS method was used for all chemicals tested. Some of the test substances were applied in concentrations lower than 100%, because they were too viscous to spread homogeneously over the test area. The final concentration and vehicle for intradermal and topical induction are given in Table 3.

Challenge. 2 weeks after topical application, the animals were tested with the induction chemical. The patch test area was occluded for 24 h and was read approximately 4 h after the patches had been removed. The reactions were evaluated blind by one of us (B.B.) and an assistant chose the cages of the animals at random.

Simultaneous reaction. In order to investigate cross sensitivity or concomitant reactions, the animals, besides being tested with the induction chemical (commercial products or its main fraction), were tested with other fractions and with other chemically-related substances. Tests were also performed with inhibitors or other chemicals which might be present in the product. 48 h after the first challenge application, the animals received a booster dose with the sensitizing chemical intradermally on the neck in the same concentration and vehicle used for intradermal induction, but without FCA (23). The animals were challenged twice, one week apart, and with a maximum of 6 test sites on the flank each time. The acrylates used for induction were also retested in the second challenge

procedure. The tests were read at the second challenge, as at the first, 4 h after the patches had been removed.

If none or only a few animals showed positive test results, the GPM-test procedure was repeated once more and with various induction concentrations. The chemicals used for challenge and their concentration and vehicles are shown in Tables 4 - 12. The test substances with petrolatum as vehicle were applied in an approximate amount of 0.015 g, and the substances with acetone or ethanol as vehicle in an amount of 0.02 ml. In all challenge applications, Finn Chambers[®] (Epitest Ltd OY, Helsinki, Finland) were used.

Controls. At the same time as the animals in the experimental group were sensitized, 15 control animals were exposed intradermally to FCA and vehicle (olive oil). The SLS procedure was used in the control animals and with petrolatum for the topical induction. When the sensitized animals were challenged, the control animals were patch tested with the same chemicals and the same concentrations. As a booster dose, the control animals, received olive oil intradermally.

2 parameters were measured in estimating sensitization: the proportion of animals sensitized and the intensity of the reaction.

The intensity of the reactions was evaluated according to the scale proposed by Magnusson & Kligman (22) with some modification (23): no visible change = 0; very faint non-confluent erythema = 0.5; confluent erythema = 1.0; erythema and slight edema = 1.5; erythema and moderate edema = 2.0; erythema and marked edema = 2.5; intense erythema and marked edema = 3.0. From this, the mean response is calculated by summation of the numerical readings of the challenge response from the various animals and dividing this sum by the total number of readings, including the negatives.

The proportion of animals sensitized was evaluated by counting the number of animals with only evident erythema and/or edema, (≥ 1.0), which was regarded as an allergic response.

The test substances can be assigned according to the % of animals sensitized, to 1 of 5 classes, ranging from a weak (grade I) to an extreme (grade V) sensitizer (22).

RESULTS

HPLC analysis

Of all the acrylates analysed, only 1 product, BIS-PMA, was sufficiently pure. All the others showed different degrees of impurity. However, the main fractions were easy to recognize in all acrylates and to collect for further testing. All main fractions were controlled by NMR to confirm the chemical structure. The commercially used BIS-GMA could be resolved into 3 different components. By means of the NMR-method, the corresponding peaks have been identified as linear BIS-GMA and branched BIS-GMA (peaks 2 and 3 in Fig. 2). NMR analyses showed that the small peak (peak 5) in the HPLC-chromatogram (Fig. 2) represents the monomethacrylate derivative, BIS-GMMA.

GPM-tests

Epoxy diacrylates. The test results in animals sensitized with Epoxy acrylate DOW (commercial product) are shown in Table 4. Of 15 animals tested, 8 were positive to the whole product at the first challenge (53%). 7 animals reacted to the main fraction of the product (47%). Tests with another brand of epoxy diacrylate (Epicote DRH-370) gave 2 (13%) positive animals. Challenge a week later showed 14 (93%) positive animals both to the whole product and to the main fraction. 1 animal was also positive when tested with the commercial product of BIS-EMA (DIACRYL 101). No positive reactions were seen to BIS-PMA, BIS-GMA, BIS-MA, epoxy resin MW 340, acrylic acid or hydroquinone.

Of those animals sensitized with the main fraction of Epoxy acrylate DOW, 9 of 15 (60%) were positive to the main fraction in the first challenge, and in the second challenge, 14 animals (93%) reacted. Tests with the whole product gave 14 positive animals (93%). The commercial product of BIS-EMA (DIACRYL 101) gave positive responses in 3 animals (20%). None reacted to BIS-PMA, BIS-GMA, BIS-MA or epoxy resin MW 340 (Table 5 and 12).

The challenge reactions in animals sensitized with another brand of epoxy diacrylate Epicote acrylate DRH-370 are shown in Table 6. 11 positive tests were seen in the first challenge (73%) and all 15 animals reacted at the second challenge (100%) to the whole product. 15 animals reacted when challenged with the whole product Epoxy acrylate DOW, and 12 (80%) to the main fraction. Tests with the whole product BIS-GMA gave 3 (20%) positive reactions. None were seen

when tested with BIS-PMA, BIS-EMA (DIACRYL 101), the main fraction of BIS-GMA, BIS-MA, epoxy resin MW 340, acrylic acid or hydroquinone.

BIS-PMA. Challenge reactions in animals sensitized with BIS-PMA with 3 different concentrations for intradermal induction were all negative. The GPM-test procedure was repeated twice with each intradermal concentration. None of the other acrylates tested was positive (Table 12).

BIS-EMA. Of the 15 animals sensitized with the commercial product BIS-EMA (DIACRYL 101) 5 reacted positively (33%) in the first and second challenge. 3 (20%) and 2 (13%) animals also reacted to the main fraction of BIS-EMA in the first and second challenges, respectively. Tests with the commercial product from another brand (Ancomer Ltd) gave 3 (20%) positive animals of the 5 positive to DIACRYL 101, 2 (13%) positive reactions were seen when tested with Epoxy acrylate DOW (main fraction) and 1 (7%) when tested with the whole product Epicote DRH-370. No reactions were seen to BIS-PMA, BIS-GMA, BIS-MA and epoxy resin MW 340 (Table 7).

Challenge reactions to the main fraction in animals sensitized with the main fraction of BIS-EMA (DIACRYL 101) showed 8 (53%) positive animals in the first challenge and 11 (73%) in the second. Only 2 (13%) animals reacted to the whole product in challenges I and II. No animals reacted to BIS-EMA from another brand (Ancomer Ltd) and none when tested with Epoxy acrylate DOW, Epicote DRH-370, BIS-PMA, BIS-GMA or BIS-MA (Table 8 and 12).

BIS-GMA. 13 (87%) of the 15 animals sensitized with the commercial product BIS-GMA (3 M) reacted when challenged with the whole product both in the first and second challenges. Tests with the different fractions of BIS-GMA, obtained by HPLC, showed that fraction 1 (Fig. 2) gave 8 (53%) positive animals. All the other fractions gave no reactions. Positive reactions in 13 (87%) animals to the whole product and 11 animals (73%) to the main fraction of Epoxy acrylate DOW were observed. 2 (14%) of the animals reacted to BIS-PMA. Challenge with epoxy resin MW 340 gave 12 (80%) positive reactions. Tests with BIS-EMA (DIACRYL 101), BIS-MA, methacrylic acid, triphenyl antimony, triphenylphosphine and hydroquinone were all negative (Table 9 and 12).

BIS-MA. Sensitization with BIS-MA (Aldrich, commercial product) showed that 8 (53%) and 10 (67%) of the animals reacted to the whole product BIS-MA.

Repeated GPM test procedure gave 12 (80%) positive tests (Challenge III) to the whole product and 14 (93%) to the main fraction, and 7 (47%) animals reacted to the pre-fraction. 4 (27%) animals reacted to epoxy resin MW 340. None reacted to epoxy acrylate DOW, BIS-PMA, BIS-EMA (DIACRYL 101), BIS-GMA, bisphenol A, glycidyl methacrylate or hydroquinone. (Table 10).

The results in animals sensitized to the main fraction of BIS-MA are shown in Table 11. 12 (80%) reacted to the main fraction of BIS-MA in the first challenge and all 15 (100%) reacted when rechallenged a week later. 7 (47%) reacted to the whole product and 11 (73%) to the pre-fraction. No positive animals were seen when tested with Epoxy acrylate DOW, BIS-PMA, BIS-EMA (DIACRYL 101), BIS-GMA, bisphenol A, epoxy resin MW 340 or methacrylic acid (Table 12).

DISCUSSION

The production of thermoset resins of the epoxy acrylate type for use as dental fillings was first described more than 20 years ago (2). In spite of increasing use of epoxy acrylates in the last decade in other areas like coatings or printing inks, only a few reports of contact allergy have been published.

Emmet & Kominsky (16) described 8 men, employed in the manufacture of ultra-violet cured inks, with allergic contact dermatitis. 3 of them had positive patch tests to epoxy acrylate. One was patch tested with 4 different commercially available epoxy acrylates and reacted only to 2. The authors suggested that variations in chain length between the monomers were of importance in the allergic reactions. In an earlier report (18), 6 patients were described who developed dermatitis while working with ultraviolet curing inks in 4 printing plants. 2 of the men were allergic to the epoxy acrylate. Nethercott (19) described a printer who developed allergic contact dermatitis due to the epoxy acrylates in the printing inks. The patient reacted when patch tested with 3 of the 4 epoxy acrylates but not to epoxy resin. The ink manufacturer stated that the epoxy acrylate, to which the patient did not react, had another chemical structure, characterized by more side chains. This may explain the failure of cross reactivity.

Of the 2 brands of epoxy diacrylate tested here, up to 93% of the guinea pigs were sensitized to Epoxy acrylate DOW and all animals to Epicote DRH-370. The classification of epoxy acrylates as extreme sensitizers is in agreement with earlier investigations when 90% of the guinea pigs were positive to another brand

of epoxy acrylate (24).

GPM-test performed by Nethercott (19) also confirms the sensitizing capacity of epoxy acrylates. He investigated 6 brands of epoxy acrylates and found that between 40% and 100% of the animals became sensitized to the products. No reaction to epoxy resin was noted.

The HPLC analysis of the 2 brands of epoxy acrylates tested in this study showed many impurities. However, after purification and testing with the main fraction of the Epoxy acrylate DOW, the same number of animals (93%) were positive as to the whole product. The chemical structure of the main fraction was controlled by NMR. No positive reactions were seen to epoxy resin MW 340 or acrylic acid, possible residues from the manufacturing process. Hydroquinone, which might have been added to the end product gave no reaction. According to the data sheet, the epoxide content in Epicote acrylate DRH370 is up to 0.02 e.q/100 g (25). Our results are in agreement with Nethercott's (19) and our earlier findings (18), that the sensitizing capacity of epoxy acrylate seems to be related to the entire molecule and not to the epoxy component or contaminants.

In spite of repeated test procedures with various concentrations for induction and challenge, we were not able to sensitize guinea pigs to BIS-PMA. Thus BIS-PMA seems to have a minimal or very low sensitizing potential. The HPLC-analysis of the batch showed a "pure" substance. To our knowledge, BIS-PMA is used in only one dental composite material on the market (Nimetic Composite Dental Restorative, ESPE GmbH, W. Germany) (6). Whether BIS-PMA is used as a prepolymer in ultraviolet inks or coatings is not known but, because of its low allergenic potential, it would be a good alternative to the other acrylates based on bisphenol A or epoxy resin, providing good technical properties.

One third of the animals sensitized with a commercial product of BIS-EMA (DIACRYL 101) became allergic, and when induction was performed with the main fraction, up to 73% reacted. This is in agreement with an earlier investigation in which 40% of the guinea pigs exposed to "bisphenol A dimethacrylate" became sensitized (24). "Bisphenol A dimethacrylate" and BIS-EMA have the same chemistry but are of different brands. None of the animals sensitized to BIS-EMA (DIACRYL 101) reacted to epoxy resin MW 340. BIS-EMA (DIACRYL 101) can be classified as a moderate sensitizer (22). Some impurities were demonstrated by HPLC analysis of the BIS-EMA batch tested. The chemical structure of the main fraction was checked by NMR. Apart from being used in radiation-curable inks and coatings. BIS-EMA is partly the monomer in some dental resins and composite materials (5,6).

BIS-GMA is the resin matrix in almost all of the commercially available composite restorative materials used today (26). Polymerization of these materials is either activated chemically (e.g. by benzoyl peroxide) or by exposure to ultraviolet radiation and an initiator (e.g. benzoin methyl ether). The major advantage of the UV-cured system is that it offers the dentist unlimited working time.

Few reports about contact allergic reactions to BIS-GMA have been published. Nathanson & Lochart (27) described a woman with a contact allergic reaction to a composite dental material confirmed by patch testing. She was also patch test positive to other products including epoxy resin. They suggested that a possible sensitizer could be the BIS-GMA monomer in the composite matrix. Niinimäki et al (20) reported a woman with edema and vesiculation on the lips and perioral skin after visiting her dentist. She had a positive patch test to the filling material used and to epoxy resin. The authors suggested that the positive reaction to epoxy resin was due to a cross-reaction to BIS-GMA, the main compound in the composite material used.

During a period of one year, 702 patients with suspected contact allergy to dental materials have been tested in various dermatology departments in Sweden with the "dental screening tray" (28). Two reacted to BIS-GMA, and they were also positive to epoxy resin in the standard test series. 2 epoxy-positive patients were also tested with BIS-GMA and 1 was positive (29).

The commercially used BIS-GMA can be resolved into 3 different components by qualitative HPLC-analysis. By means of NMR, 2 of the components have been identified by us and by others (6, 30) as a linear BIS-GMA and a branched BIS-GMA (peak 2 and 3 in Fig. 2). Mass spectrometry also shows that the small peak (peak 5) in the HPLC-chromatogram (Fig. 2) represents the monomethacrylate derivative BIS-GMMA (6). A fourth isomer, the double branched BIS-GMA should also be expected in the mixture at a quantity of about 2% (6). This isomer was, however, not detected in our chromatogram. In all BIS-GMA products analysed, it is always in the same proportion (3:1) of linear and branched isomers (6). This indicates that the branched BIS-GMA isomer is not intentionally added to the monomer mixture, but that it should be considered as a side product in the synthesis of the BIS-GMA monomer (30). Separation of the linear BIS-GMA

monomer from the branched one would be rather difficult on a large scale (30). BIS-GMA can be synthesized in two ways: (i) by the reaction of glycidyl methacrylate with bisphenol A or (ii) by the reaction of methacrylic acid with diglycidyl ether of bisphenol A (epoxy resin). In either way, the reaction of the 2 compounds is accompanied by a ring opening of the epoxide group. Both epoxide moieties of the molecular react independently of one another, and therefore the different BIS-GMA isomers are formed (Fig. 1).

Of those guinea pigs sensitized to the commercial product of BIS-GMA (3 M), 87% reacted positively when tested with the whole product. Only fraction 1 (free from linear and branched BIS-GMA and BIS-GMMA, (Fig. 2) of the different fractions of BIS-GMA tested, gave positive reactions in 53% of the animals. Thus, the linear and the branched isomer or BIS-GMMA (fraction 4) did not seem to have any allergenic properties. The challenge procedure was repeated with various test concentrations. Tests with epoxy resin MW 340 yielded 80% positive animals. Thus it seems that only the impurity fraction (fraction 1) of the BIS-GMA product possesses allergenic potential. Epoxy resin MW 340 has its peak in the BIS-GMA chromatogram in the same area as fraction 1. Whether any residual epoxy resin monomer is left unreacted or formed in the synthesis of the BIS-GMA monomer is uncertain. The presence of epoxy resin MW 340 is a probable explanation of the high number of positive epoxy tests in the BIS-GMA sensitized animals.

Compounds such as methacrylic acid, are known to be present in BIS-GMA resins, primarily because of an incomplete synthesis reaction. Traces of compounds, such as triphenyl antimony and triphenylphosphine, or their oxidized derivatives, may also be present (7). Tests with methacrylic acid, triphenyl antimony and triphenylphosphine and hydroquinone were all negative.

Induction with the commercial product of BIS-MA gave up to 80% positive animals to the whole product, 93% to the main fraction, and 47% to the impurity fraction (pre-fraction). Of those animals sensitized to the main fraction of BIS-MA, all reacted to the main fraction at the second challenge. Tests with the whole product gave 47% positive animals, and with the impurity fraction as many as 73%. The impurity fraction was free from the main fraction. Thus, it seems that not only the main fraction but also the impurity fraction (pre-fraction) contains an allergen, and cross reaction between the two substances occurs.

One fourth (27%) of the animals sensitized to the whole product of BIS-MA reacted to epoxy resin MW 340, but none of the animals sensitized with the main fraction. It is probable that small amounts of any residual epoxy resin present in the pre-fraction of the BIS-MA product, sensitized some animals. These reactions are not explained by the presence of bisphenol A, glycidyl methacrylate or hydroquinone, which were all negative.

Simultaneous reaction pattern. Table 12 shows that 20% of the animals sensitized to the main fraction of Epoxy acrylate DOW, reacted to the commercial product of BIS-EMA (DIACRYL 101). Of the 15 animals sensitized to the commercial product of BIS-EMA (DIACRYL 101), 2 (13%) reacted to the main fraction of Epoxy acrylate DOW (Table 7). However, none of the animals sensitized to the main fraction of BIS-EMA (DIACRYL 101) reacted when tested with the main fraction of Epoxy acrylate DOW (Table 8).

Thus, a component in the impurity part of the commercial BIS-EMA (DIACRYL 101) is structurally related to the epoxy diacrylate molecule and occurs in a concentration sufficient to sensitize some animals.

Of the animals sensitized with the commercial product of BIS-GMA, 73% reacted to the main fraction of Epoxy acrylate DOW and 14% to BIS-PMA. It seems that concomitant reactions occur between the products. However, the BIS-GMA sensitized animals reacted only to fraction 1. It is probable that fraction 1 of the BIS-GMA product contains small amounts of epoxy diacrylate and BIS-PMA or similar substances which can cross react with these. It is also possible that the BIS-PMA product contains undetected impurities.

Neither the animals we tried to sensitize with BIS-PMA, nor the BIS-MA sensitized animals, reacted when challenged with the other acrylates tested.

It is known that a dermatitis on one part of the body increases the reactivity of the unaffected skin at other sites the so called excited skin syndrome, angry back syndrome or status eczematicus. When challenged with more than one compound, a positive reaction can influence the test response of the others, and thus give false positive tests. Application of only one substance in successive challenges with more than a week apart might be the proper way to investigate a concomitant reaction pattern. It is possible in humans, but in the guinea pigs,

wrapping with adhesive tape and repeated tests will also interfere with the test response.

Inhibitors or stabilizers are ingredients in any formulation involving free radical chemistry. They retard or prevent polymerization until cure is desired. From information obtained from the manufacturers of the acrylates tested, hydroquinone was the inhibitor mostly used. p-Methoxyphenol is also a common inhibitor (31).

Few reports on patch testing with hydroquinone in acrylate-sensitive patients are published. Magnusson (32) sensitized a few patients to methyl methacrylate. 2 of them had also been sensitized to hydroquinone used in the monomer. Magnusson noted that 5 out of 8 volunteers exposed to hydroquinone stabilized methyl methacrylate monomer became sensitive to hydroquinone. He suggested that methyl methacrylate somehow facilitates or predisposes to the development of the hydroquinone sensitivity. Magnusson & Mobacken (33) found negative test results with hydroquinone in 7 workers sensitized to an acrylic sealant. Kaaber & Thulin (34) observed positive reactions to hydroquinone in a patient with an acrylic denture.

In a systemic analysis of the sensitizing potential of mono(meth)acrylates and di(meth)acrylates in guinea pigs, van der Walle et al (31, 35, 36) found concomitant sensitization to hydroquinone and/or p-methoxyphenol present in the acrylic monomers. The inhibitors had no influence on the sensitizing potential of the acrylic monomers (37). Induction and challenge with the inhibitors showed hydroquinone and p-methoxyphenol to be moderate sensitizers and cross reactions between the 2 compounds occurred (31). None of the animals sensitized to the various commercial acrylate products in our investigation were found to be allergic to hydroquinone. The challenge concentration of hydroquinone was 0.1% w/w in ethanol. A concentration of 0.5% and higher was found to be irritant. Van der Walle et al (31) used a challenge concentration of 1 M (approx. 10%) in Aramek (2 parts methyl ethyl ketone + 1 part arachis oil v/v). A too high concentration and thus false positive tests might explain the different results between our study and theirs.

We have added thymol to the two fractions of BIS-MA to prevent polymerization. Test with thymol have not been performed because, in various animal experiments no sensitizing potential has been detected (31, 38).

This investigation has shown that epoxy diacrylate, BIS-EMA and BIS-MA are strong sensitizers, while the linear fraction of BIS-GMA and its isomers and BIS-PMA have none or low sensitizing capacity. The chemical structure of linear BIS-GMA is that of an epoxy dimethacrylate. A decrease in sensitizing potential with the introduction of a methyl group at the α -carbon atom in the terminal acrylic part has been shown in other investigations (35, 39). However, BIS-EMA sensitized up to 73% and BIS-MA all animals in spite of the methacrylated end groups. No test reactions were seen in the BIS-PMA sensitized animals. The BIS-PMA molecule has a linear structure with 3 methylene groups in each moiety. Thus it seems that 3 or more methylene groups in the molecular chain diminish the allergenicity. The impurities in the BIS-GMA and BIS-MA batches seem to have higher allergenic potential. Our results emphasize the importance of a pure substance when discussing allergenicity and cross reaction.

The patent literature describes the utility of epoxy acrylate type products in dentistry, photoresists and ultraviolet or electron beam cured printing inks or coatings. They are expected to be used in a wide variety of applications because of their reactivity and the versatility that can be designed into the backbone. Since different products exhibit different degrees of potential hazard, specific recommendations for handling should be obtained from the manufacturers. We suggest that manufacturers, if technically possible, formulate an epoxy acrylate type of resin based on a sufficiently long chain length with methacrylated end groups, and with a high degree of purity, to avoid unexpected contact allergic reactions to products on the market.

ACKNOWLEDGEMENTS

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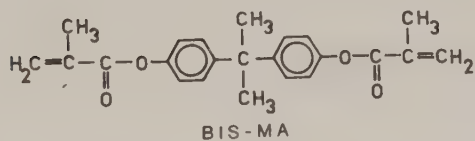
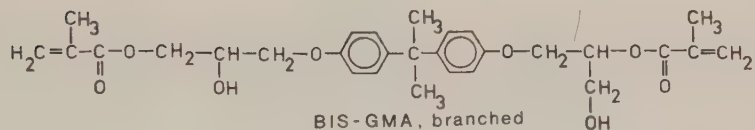
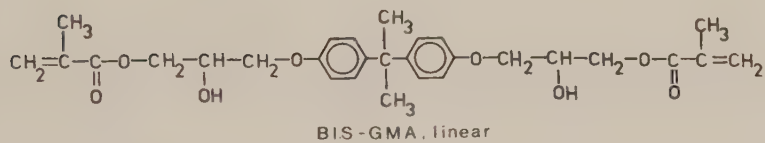
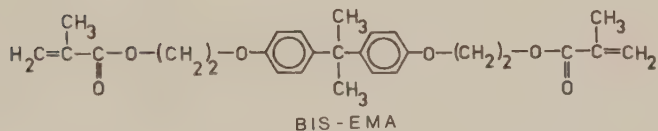
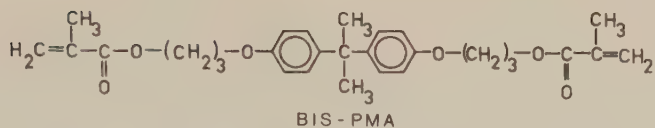
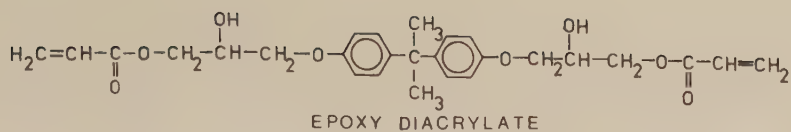


Fig. 1. Chemical structure of the acrylates tested based on bisphenol A or epoxy resin.

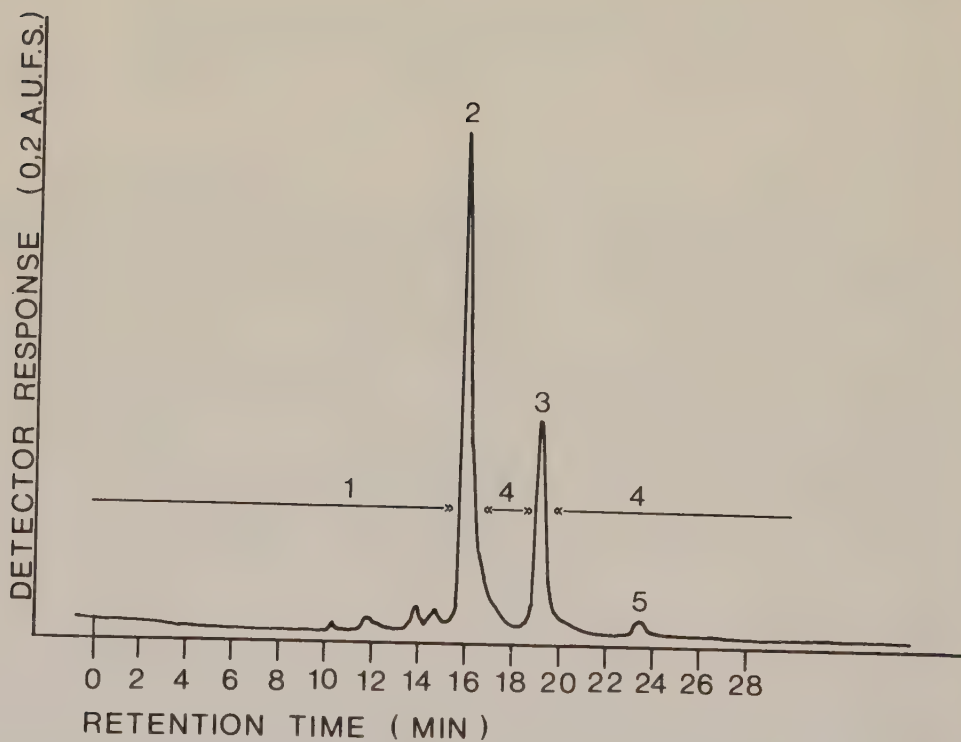


Fig. 2. Example of a chromatogram showing the separation of BIS-GMA (3 M) in different fractions by HPLC. The identified peaks are: 2 = BIS-GMA, linear, 3 = BIS-GMA, branched, 5 = BIS-GMMA, bisphenol A diglycidyl monomethacrylate.

Table 1. CONDITIONS FOR ANALYTICAL SEPARATIONS

	Epoxy acrylate DOW (DOW Chemicals, Switzerland)	BIS-PMA (ESPE GmbH, W.Germany)	BIS-EMA (Diacyrl 101) (AKZO Chemie, The Netherlands)	BIS-GMA (3 M Company, USA)	BIS-MA (Aldrich-Europe, Belgium)
column	Spherisorb	1) Nucleosil C-18 2) Nucleosil CN	Spherisorb	1) Partisil M9 2) Nucleosil C-18	Nucleosil C-18
mobile phase	acetonitrile/ water (60:40)	1) acetonitrile/ water (90:10) 2a)acetonitrile/ water (60:40) b)hexane/2-propanol (99.9:0.1)	acetonitrile/ water (75:25)	1)dichlorome- thane/2-propanol (97:3) 2)acetonitrile/ water (75:25)	acetonitrile/ water (90:10)
flow rate (ml/min)	1	1) 0.8 2) 1	1	1) 1.2 2) 1	0.8
wavelength for UV-detection (nm)	280	1) 230, 254, 267, 290 2) 254, 280	278	267, 290	267

Table 2. CONDITIONS FOR SEMI-PREPARATIVE SEPARATIONS

	Epoxy acrylate DOW (DOW Chemical, Switzerland)	BIS-EMA (Diacryl 101) (AKZO Chemie, The Netherlands)	BIS-GMA (3 M Company, USA)	BIS-MA (Aldrich-Europe, Belgium)
column	Partisil ODS	Partisil ODS	Partisil M9	Merck RP 18
mobile phase	acetonitrile/ water (70:30)	acetonitrile/ water (75:25)	dichloromethane/ methanol (97:3)	acetonitrile/ water (90:10)
flow rate (ml/min)	2	2.5	1	1.3
wavelength for UV-detection	254	278	290	267
fractions collected	main fraction	main fraction	1) impurity 2) BIS-GMA linear 3) BIS-GMA branched 4) impurity	1) impurity (pre- fraction 2) main fraction
inhibitor				thymol (main fraction) thymol+hydroquinone (impurity pre-fraction)

Table 3. CONCENTRATIONS USED FOR INTRADERMAL AND TOPICAL INDUCTION

Chemical	Trade name	Part of the product used	Molecular weight	Intradermal concentration in olive oil (% v/w)	Topical concentration in pet (% v/w)
Epoxy diacrylate	XD-8008.04 (DOW)	whole main fraction		5	100
			484	5	100
Epoxy diacrylate	Epicote DRH-370 (Shell)	whole main fraction		5	100
			484	N.D.	N.D.
BIS-PMA	BIS-PMA (ESPE)	whole main fraction		20, 10, 5	100
			480	N.D.	N.D.
BIS-EMA	Diacryl 101 (AKZO)	whole main fraction		10	100
			452	5a	50
BIS-GMA	BIS-GMA (3 M)	whole main fraction		20, 10	100
			512	N.D.	N.D.
BIS-MA	BIS-MA(Aldrich)	whole main fraction		10, 10 ^a	50
			364	10 ^a	25

Before topical induction all animals were pretreated with SLS

^avehicle = olive oil/acetone, 10:1 N.D. = not done

Table 4. CHALLENGE REACTIONS IN ANIMALS SENSITIZED WITH EPOXY ACRYLATE DOW (COMMERCIAL PRODUCT)

Chemical	Challenge conc. and vehicle	number	Challenge I		number	Challenge II	
			%pos.	mean response		%pos.	mean response
Epoxy acrylate (DOW)							
whole product	10% pet	8	53	0.90	14	93	1.73
main fraction	5% pet	7	47	0.63	14	93	1.60
Epikote DRH-370							
whole product	10% ac	2	13	0.20	-	-	-
BIS-PMA	10% pet	0	0	0	-	-	-
BIS-EMA (DIACRYL 101)							
whole product	10% pet	-	-	-	1	7	0.13
BIS-GMA							
whole product	10% pet	-	-	-	0	0	0
BIS-MA							
whole product	10% pet	0	0	0	-	-	-
epoxy resin MW 340	1% pet	0	0	0	-	-	-
acrylic acid	0.5% ac	-	-	-	0	0	0
hydroquinone	0.1% alc	-	-	-	0	0	0

The number indicates the positive animals of 15 tested. All 15 control animals were negative for all chemicals.

- = not tested; pet. = petrolatum; ac. = acetone; alc. = ethanol 99%.

Table 5. CHALLENGE REACTIONS IN ANIMALS SENSITIZED WITH THE MAIN FRACTION OF EPOXY ACRYLATE DOW

Chemical	Challenge conc. and vehicle	number	Challenge I %pos.	mean response	number	Challenge II %pos.	mean response
Epoxy acrylate DOW							
main fraction	5% pet	9	60	0.80	14	93	1.43
whole product	10% pet	-	-	-	14	93	1.50
BIS-PMA	25% ac	-	-	-	0	0	0
BIS-PMA	10% pet	0	0	0	-	-	-
BIS-EMA (DIACRYL 101)							
whole product	10% pet	3	20	0.27	-	-	-
BIS-GMA (3 M)							
linear fraction(2)	5% pet	0	0	0	-	-	-
linear fraction	10% ac	-	-	-	0	0	0
BIS-MA							
whole product	10% pet	0	0	0	-	-	-
epoxy resin MW 340	1% pet	0	0	0	-	-	-

The number indicates the positive animals of 15 tested. All 15 control animals were negative for all chemicals.

- = not tested; pet. = petrolatum; ac. = acetone.

Table 6. CHALLENGE REACTIONS IN ANIMALS SENSITIZED WITH EPIKOTE ACRYLATE DRH-370

Chemical	Challenge conc. and vehicle	number	Challenge I %pos.	mean response	number	Challenge II %pos.	mean response
Epicote acrylate DRH-370 whole product	10% pet.	11	73	0.86	15	100	1.53
Epoxy acrylate DOW whole product	10% pet	-	-	-	15	100	1.23
main fraction	5% pet	-	-	-	12	80	0.93
BIS-PMA	10% pet	-	-	-	0	0	0
BIS-EMA (DIACRYL 101) whole product	10% pet	0	0	0	-	-	-
main fraction	5% pet	0	0	0	-	-	-
BIS-GMA whole product	10% pet	-	-	-	3	20	0.33
main fraction	5% pet	-	-	-	0	0	0
BIS-MA whole product	10% pet	0	0	0	-	-	-
epoxy resin MW 340	1% pet	-	-	-	0	0	0
acylic acid	0.5% ac	0	0	0	-	-	-
hydroquinone	0.1% alc	0	0	0	-	-	-

The number indicates the positive animals of 15 tested. All 15 control animals were negative for all chemicals.

- = not tested; pet. = petrolatum; ac. = acetone; alc. = ethanol 99%.

Table 7. CHALLENGE REACTIONS IN ANIMALS SESITIZED WITH BIS-EMA (DIACRYL 101, COMMERCIAL PRODUCT)

Chemical	Challenge conc.and vehicle	number	Challenge I %pos.	mean response	number	Challenge II %pos.	mean response
BIS-EMA (DIACRYL 101)							
whole product	10% pet	5	33	0.40	5	33	0.43
main fraction	5% pet	3	20	0.23	2	13	0.17
BIS-EMA(Ancomer Ltd)							
whole product	10% pet	-	-	-	3	20	0.20
Epicote DRH-370							
whole product	10% pet	-	-	-	1	7	0.07
Epoxy acrylate DOW							
main fraction	5% pet	2	13	0.13	0	0	0
BIS-PMA	10% pet	0	0	0	-	-	-
BIS-GMA (3 M)							
whole product	10% pet	-	-	-	0	0	0
BIS-MA							
whole product	10% pet	-	-	-	0	0	0
epoxy resin MW 340	1% pet	0	0	0	-	-	-
hydroquinone	0.1% alc	0	0	0	-	-	-

The number indicates the positive animals of 15 tested. All 15 control animals were negative for all chemicals tested.

- = not tested; pet. = petrolatum; alc.= ethanol 99%.

Table 8. CHALLENGE REACTIONS IN ANIMALS SENSITIZED WITH THE MAIN FRACTION OF BIS-EMA (DIACRYL 101)

Chemical	Challenge conc. and vehicle	number	%pos	mean response	number	%pos.	mean response
BIS-EMA (DIACRYL 101)							
main fraction	5% pet	8	53	0.73	11	73	0.83
whole product	10% pet	2	13	0.23	2	13	0.23
BIS-EMA (Ancomer Ltd)							
whole product	10% pet	-	-	-	0	0	0
Epicote DRH-370							
whole product	10% pet	-	-	-	0	0	0
Epoxy acrylate DOW							
main fraction	5% pet	0	0	0	-	-	-
whole product	10% pet	0	0	0	-	-	-
BIS-PMA	10% pet	0	0	0	-	-	-
BIS-GMA (3 M)							
whole product	10% pet	-	-	-	0	0	0
BIS-MA							
whole product	10% pet	-	-	-	0	0	0

The number indicates the positive animals of 15 tested. All 15 control animals were negative for all chemicals tested.

- = not tested; pet = petrolatum.

Table 9. CHALLENGE REACTIONS IN ANIMALS SENSITIZED WITH BIS-GMA
(3 M,COMMERCIAL PRODUCT)

Chemical	Challenge conc.and vehicle	number	Challenge I %pos. mean response		number	Challenge II %pos. mean response	
BIS-GMA							
whole product	10% pet	13	87	1.17	13	87	1.17
fraction 1	5% pet	8	53	0.83	-	-	-
fraction 2 (linear)	5% pet	0	0	0	-	-	-
fraction 3 (branched)	5% pet	0	0	0	-	-	-
fraction 4	5% pet	0	0	0	-	-	-
Epoxy acrylate DOW							
whole product	10% pet	-	-	-	13	87	1.33
main fraction	5%	-	-	-	11	73	0.90
BIS-PMA	10 %	2	14	0.17	-	-	-
BIS-EMA(DIACRYL 101)							
whole product	25% ac	-	-	-	0	0	0
BIS-MA							
whole product	10% pet	0	0	0	-	-	-
methacrylic acid	1% ac	-	-	-	0	0	0
triphenyl anitmony	1% ac	-	-	-	0	0	0
triphenylphosfine	1% ac	-	-	-	0	0	0
hydroquinone	0.1% alc	0	0	0	-	-	-
epoxy resin MW 340	1% pet	12	80	1.17	-	-	-

The number indicates the positive animals of 15 tested. All control animals were negative for all chemicals tested.

- = not tested; pet. = petrolatum; ac. = acetone; alc. = ethanol 99%.

Table 10. CHALLENGE REACTIONS IN ANIMALS SENSITIZED WITH BIS-MA (ALDRICH, COMMERCIAL PRODUCT)

Chemical	Challenge conc.and vehicle	Challenge I			Challenge II			Challenge III		
		n	%	m	n	%	m	n	%	m
Chemical	Challenge conc.and vehicle	n	%	m	n	%	m	n	%	m
BIS-MA										
whole product	10% pet	8	53	0.93	10	67	1.03	12	80	1.30
pre fraction 1	5% pet	-	-	-	-	-	-	7	47	0.53
Main fraction 2	5% pet	-	-	-	-	-	-	14	93	1.57
Epoxy acrylate DOW										
whole product	10% pet	-	-	-	0	0	0	-	-	-
main fraction	5% pet	-	-	-	0	0	0	-	-	-
BIS-PMA	10% pet	0	0	0	-	-	-	-	-	-
BIS-EMA(DIACRYL 101)										
whole product	10% pet	0	0	0	-	-	-	-	-	-
main fraction	5%	0	0	0	-	-	-	-	-	-
BIS-GMA (3 M)										
whole product	10% pet	-	-	-	-	-	-	0	0	0
main fraction	5% pet	-	-	-	-	-	-	0	0	0
bisphenol A	1% pet	-	-	-	0	0	0	-	-	-
epoxy resin MW 340	1% pet	-	-	-	-	-	-	4	27	0.2
glycidyl methacrylate	0.5% ac	0	0	0	-	-	-	-	-	-
hydroquinone	0.1% alc	0	0	0	-	-	-	-	-	-

The number indicates the positive animals of 15 tested. All control animals were negative for all chemicals tested.

- = not tested; pet. = petrolatum; alc. = ethanol 99%.

n = number; % = pos.; m = mean response.

Table 11. CHALLENGE REACTIONS IN ANIMALS SENSITIZED WITH THE MAIN FRACTION OF BIS-MA (ALDRICH)

Chemical	Challenge conc. and vehicle	number	%pos.	mean response	number	%pos.	mean response
BIS-MA							
main fraction 2	5% pet	12	80	1.40	15	100	1.63
pre fraction 1	5% pet	11	73	0.93	-	-	-
whole product	10% pet	7	47	0.77	-	-	-
Epoxy acrylate DOW							
main fraction	5% pet	-	-	-	0	0	0
BIS-PMA	10% pet	0	0	0	-	-	-
BIS-EMA (DIACRYL 101)							
whole product	10% pet	-	-	-	0	0	0
BIS-GMA (3M)							
main fraction	5% pet	-	-	-	0	0	0
bisphenol A	1% pet	0	0	0	-	-	-
epoxy resin MW 340	1% pet	0	0	0	-	-	-
methacrylic acid	1% pet	-	-	-	0	0	0

The number indicates the positive animals of 15 tested. All control animals were negative for all chemicals tested.

- = not tested; pet. = petrolatum

Table 12. SIMULTANEOUS REACTION PATTERN IN ANIMALS SENSITIZED AND CHALLENGED WITH ACRYLATES BASED ON BISPHENOL A OR EPOXY RESIN

Challenged with Sensitized to	Epoxy acrylate DOW	BIS-PMA ^a	BIS-EMA (DIACRYL 101)	BIS-GMA ^a	BIS-MA
Epoxy acrylate DOW	14	0 ^a	3 ^a	0	0 ^a
BIS-PMA ^a	0	0 ^a	0	0	0 ^a
BIS-EMA (DIACRYL 101)	0	0 ^a	11	0 ^a	0 ^a
BIS-GMA ^a	11	2 ^a	0 ^a	13 ^a	0 ^a
BIS-MA	0	0 ^a	0 ^a	0	15

The number indicates the maximum number of positive animals.

^aInduction or challenge performed with the whole product, otherwise with the main fraction of the acrylate.

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VI

CONTACT ALLERGY TO 2-HYDROXYPROPYL METHACRYLATE (2-HPMA)

IN AN ULTRAVIOLET CURABLE INK

Bert Björkner

Contact Allergy to 2-Hydroxypropyl Methacrylate (2-HPMA) in an Ultraviolet Curable Ink

BERT BJÖRKNER

Department of Occupational Dermatology, Department of Dermatology in Lund and Malmö, Department of Experimental Research, General Hospital, Malmö, Sweden

Björkner B. Contact allergy to 2-hydroxypropyl methacrylate (2-HPMA) in an ultraviolet curable ink. *Acta Derm Venereol* (Stockh) 1984; 64:

A patient working in an ink laboratory developed dermatitis on his hands. Patch testing revealed contact allergy to the ink used and to 2-hydroxypropyl methacrylate (2-HPMA), the monomer present in the ink. Guinea pig maximisation test (GPM-test) shows that 2-HPMA is a weak sensitizer. The data suggest cross-reactivity to 2-hydroxyethyl methacrylate (2-HEMA) but not to 2-hydroxypropyl acrylate (HPA) or 2-hydroxyethyl acrylate (2-HEA). *Key words:* Contact allergy; Guinea pig maximization test; Hydroquinone. (Received October 21, 1983.)

B. Björkner, Department of Occupational Dermatology, General Hospital, S-21401 Malmö, Sweden.

A patient working in an ink laboratory, formulating inks and varnishes for UV cure, developed a dermatitis on his hands. Patch testing revealed contact allergy to the ink he was working with and 2-hydroxypropyl methacrylate (2-HPMA), the monomer present in the composition. As 2-HPMA is a common acrylic monomer in UV-curable acrylic resins and other applications, its sensitizing capacity was investigated by means of the guinea pig maximization test (GPM test) (1, 2).

MATERIALS AND METHODS

Case report

A 52 year old man has been employed for 10 years in an ink laboratory, doing research work with inks and varnishes containing acrylates for ultraviolet cure. He had never had any skin problems earlier. In December 1980 he was working with an UV lamp and in the evening noticed he had swollen and red hands. He cleared on local corticosteroids. A couple of weeks later he noticed the same dermatitis in three occasions. The ink consisted of a polyesteracrylate as a polymer and 2-HPMA as a monomer. He was tested with UV-B and UV-A and photo patch tested with the standard test series and also with the ink he has been working with in a concentration from 1% w/w diluted down to 0.01% w/w in methyl ethyl ketone (MEK). He was patch tested with polyesteracrylate (Ebecryl 810, UCB, Belgium) and 2-HPMA (BDH Chemicals Ltd, England) in a concentration of 2% w/w in petrolatum. He was also patch tested with other acrylates he might have been exposed to, such as trimethylolpropane triacrylate (TMPTA, UCB, Belgium), pentaerythritol triacrylate (PETA, Svenska Lorilleux, Sweden), OTA 480 (UCB, Belgium) all in a concentration of 0.1% w/w in pet. They are all common multifunctional acrylates in UV-curing inks. Patch testing was also performed with dimethacrylates based on bisphenol A (3), such as BIS-MA (2% w/w in pet.), BIS-EMA (2% w/w in pet.), BIS-MA (2% w/w in pet.), BIS-PMA (5% w/w in pet.). He was also tested with two epoxy diacrylates (Epikote DRH-340 and Epoxy acrylate DOW), both in 5% w/w in pet. The epoxy diacrylates are diacrylates based on bisphenol A (3). He was also tested with a photoinitiator and an accelerator used in the ink. The chemistry of those substances were unknown to us.

Guinea Pig Maximization Test (GPM Test)

Chemicals. The chemicals used in the GPM test were 2-hydroxy-propyl methacrylate (2-HPMA) and 2-hydroxyethyl methacrylate (2-HEMA), manufactured by BDH Chemicals Ltd, England; 2-hydroxypropyl acrylate (2-HPA) and 2-hydroxyethyl acrylate (2-HEA), manufactured by Pfaltz & Bauer Inc., USA. Hydroquinone was purchased from Fluka AG, Switzerland. The chemical structure of the acrylates tested are given in Fig. 1. The purity of the acrylates, analysed by high performance liquid chromatography, was at least 95%.

GPM-test. The methods were in accordance with the original description of the GPM test (1, 2) and under the same condition as reported in previous studies (3). Ten guinea pigs were used as experimental animals and 10 animals served as controls. The sensitization procedure was repeated once with other guinea pigs than used in the first experiment.

Five % w/w 2-HPMA solved in a mixture of olive oil and acetone (10:1) was used for intradermal induction.

To achieve a uniform dispersion of 2-HPMA in petrolatum, only 25% w/w was used for topical induction. Pretreatment with 10% w/w sodium lauryl sulphate (SLS) in water was performed, as 25% w/w concentration did not give any irritation.

Challenge was performed with 2-HPMA (2% w/w in pet.), 2-HEMA (2% w/w in pet.), 2-HPA (0.2% w/w in pet.), 2-HEA (0.5% w/w in pet.) and hydroquinone (0.05% w/w in pet.).

RESULTS

Testing of the patient

The photopatch test was negative for the standard test series but positive for the ink used both at the irradiated and covered test sites with a test concentration of 1% and 0.1% in MEK but negative for 0.01%. Photo tests were normal for UV-A and UV-B. The standard epicutaneous patch test was negative. Tests using the different acrylates showed positive reaction only for 2-HPMA. Patch tests with the photoinitiator and accelerator were also negative.

GPM test

One animal out of 10 reacted to 2-HPMA. The same animal also reacted to 2-HEMA with same mean response (0.15) as for 2-HPMA. None of the animals reacted to 2-HPA, 2-HEA and hydroquinone. All control animals were negative for all substances tested. The repeated sensitization procedure gave the same results.

DISCUSSION

2-HPMA is a common monofunctional acrylic monomer used in UV-curable resins. Other applications where 2-HPMA might be an important ingredient are dental fissure sealents, prosthetic appliance formulations for dentistry and orthopaedic surgery, impregnated fabric articles as orthopaedic casts and in certain shoe devices.

This investigation shows that 2-HPMA is a weak sensitizer in guinea pigs. Contact sensitization in guinea pigs from 2-HPMA has, to our knowledge, not been reported earlier. The data may suggest cross-reactivity or concomitant reactivity to 2-HEMA as the same animal reacted to both 2-HPMA and 2-HEMA in both sensitizing experiments.

The patient positive to 2-HPMA was never patch tested with 2-HEMA as he declined further testing.

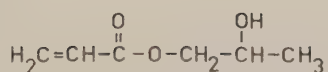
There are reports on positive patch test reactions to 2-HPMA in printers exposed to NAPP® printing plates (4, 5, 6). It is unknown if the NAPP® printing plate contains 2-HPMA.

Grimalt & Romequera (7) patch tested 45 patients with shoe dermatitis, and one of those was positive to hydroxypropyl methacrylate. If 1-HPMA or 2-HPMA was used in the patch test series, is not known. It is also unknown if the shoes the patient had used contained any hydroxypropyl methacrylate.

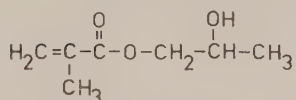
Some reports about contact allergy to 2-HEMA have been published. Malten & Bende (8) described 5 patients which developed an allergic contact dermatitis when working with photoprepolymer printing plate making procedure. Four of them were patch tested and showed positive reaction to 2-HEMA, one of the ingredients in the photoprepolymer mixture. In order to obtain information about possible cross-reacting substances 2 of the patients were also tested with ethyl methacrylate with negative results.

Parker & Turk (9) investigated 21 different acrylate and methacrylate compounds for their ability to induce contact sensitivity in the guinea pig. It was not possible to induce contact sensitivity to 2-HEMA or any other methacrylates tested using the Polak method of immunization. However, contact reactions of varying intensities were produced to all the mono-, di and triacrylates tested. Van der Walle et al. (10) investigated the sensitizing potential of 2-HEMA in guinea pigs with Freund's Complete Adjuvant Test (FCAT). All animals were positive on day 21 but negative on day 35. Jordan (11) described 7 patients which developed allergic contact dermatitis to an acrylic based adhesive tape. Five of the subjects were patch tested with positive results to 2-ethylhexyl acrylate, one of the ingredients in the adhesive tape. They were all negative to 2-ethylhexyl methacrylate. Two of the subjects were also positive to 1-HPA and 1-HPMA.

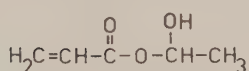
In our experiments tests with 2-HPA and 2-HEA were negative. 2-HEA has been shown to be a strong sensitizer in guinea pigs (12) thus, with introduction of a methyl group at the α -carbon atom, there seems to be a decrease in the sensitizing potential.



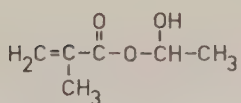
2-HYDROXYPROPYL ACRYLATE



2-HYDROXYPROPYL METHACRYLATE



2-HYDROXYETHYL ACRYLATE



2-HYDROXYETHYL METHACRYLATE

Fig. 1. Structural formula of 2-hydroxypropyl acrylate (2-HPA), 2-hydroxypropyl methacrylate (2-HPMA), 2-hydroxyethyl acrylate (2-HEA) and 2-hydroxyethyl methacrylate (2-HEMA).

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VII

SENSITIZATION POTENTIAL OF URETHANE (METH)ACRYLATES
IN THE GUINEA PIG

Bert Björkner

SENSITIZING POTENTIAL OF URETHANE (METH)ACRYLATES IN THE GUINEA PIG

Bert Björkner

Department of Occupational Dermatology, department of Dermatology in Lund and Malmö and Department of Experimental Research, General Hospital, Malmö, Sweden.

Contact Dermatitis (Accepted for publication)

Urethane (meth)acrylates are used as prepolymers in ultraviolet (UV) curable inks, varnishes and coating formulations for industrial purposes as well as resins for dental applications.

The sensitizing capacity of 3 urethane (meth)acrylates, commonly used on the market, was investigated with the "Guinea Pig Maximization Test" (GPM-test). The study shows that an aliphatic urethane acrylate was a more potent sensitizer than an aromatic one, but also more sensitizing than a methacrylated aliphatic urethane.

Key words: Guinea Pig Maximization Test - ultraviolet curable ink - urethane (meth)acrylate - sensitizing potential.

In the ultraviolet (UV) curable coating formulations, the UV-reactive prepolymers are the most essential components. Among the acrylated prepolymers reported are: Polyethers, urethanes, polyesters, alkyds, epoxies, modified epoxies, acrylics and polycaprolactones (1-6).

Of the many different types of acrylated urethanes on the market, some are based on aromatic isocyanates while others are of the aliphatic types. They can vary in functionality with acrylated or methacrylated groups and with various molecular weights. Urethane (meth)acrylates are used not only as prepolymers in UV-curable inks, varnishes and coating formulations for industrial purposes, but as resins for dental applications as well.

The aim of the present work was to investigate the sensitizing potential of some

commonly used urethane (meth)acrylates, using the "Guinea Pig Maximization Test" (7 - 8).

MATERIAL AND METHODS

The induction and challenge procedure was performed after the original description of the GPM-test (7-8) and under the same condition as in previous studies (9). Fifteen female albino guinea pigs of the Dunkin-Hartley strain, weighing 300-400 grams, were used as experimental animals and the same number as control animals.

Chemicals. The 3 urethane (meth)acrylates chosen for this study were commercial products. Two were urethane acrylates, commonly used as prepolymers in the coating industry, one of the aromatic type (Ebecryl 220) and one of the aliphatic type (Ebecryl 270). They were purchased from UCB, Belgium. The aromatic urethane acrylate (Ebecryl 220) is based on toluene diisocyanate (TDI) with a functionality of 6 and a molecular weight of 1000. The aliphatic urethane acrylate (Ebecryl 270) has the functionality of 2 and a molecular weight of 1500. Which aliphatic diisocyanate used was not disclosed by the manufacturer. The two urethane acrylates were of technical grade. The third acrylated urethane tested was an aliphatic urethane methacrylate (UEDMA), commonly used as dental composite resin. UEDMA, purchased from Ivoclar AG, Lichtenstein, has a molecular weight of 470 and a functionality of 2 with a purity of 98%.

Induction procedure. Three animals for each product were used to evaluate the optimal concentrations for intradermal and topical induction. The animals were thereafter not used in the sensitization experiments. All the urethane (meth)-acrylates were solved in a mixture of olive oil and acetone (9:1). The appropriate intradermal concentration for Ebecryl 220 and Ebecryl 270 was 1% w/w and for UEDMA 5% w/w. The topical induction concentration for all urethane (meth)acrylates was 100%. Pretreatment with 10% sodium lauryl sulphate (SLS) in petrolatum was necessary before the topical induction with Ebecryl 270 and UEDMA. Ebecryl 220 gave an appropriate irritation without pretreatment with SLS.

Challenge procedure. The challenge concentrations had previously been evaluated

on unused animals. The animals were tested with the urethane (meth)acrylate used for induction but also with the other two acrylic compounds in order to investigate simultaneous reactivity. The test concentrations chosen for Ebecryl 270 and UEDMA was 1% w/w in petrolatum and for Ebecryl 220, 0.2% w/w in petrolatum. The amount of test material was 0.015 g. Challenge was also performed with hydroquinone (0.1% w/w in alcohol) in all animals. The final concentrations for induction and challenge for the urethane (meth)acrylates tested are shown in Table 1. The test results were read and evaluated in the same manner described earlier (9).

RESULTS

Of the 15 animals sensitized to the aromatic urethane acrylate Ebecryl 220, 2 (15%) showed positive test reactions. Guinea pigs sensitized to the aliphatic urethane acrylate Ebecryl 270, 13 of 15 (87%) gave positive test response. Of the 15 animals sensitized to the aliphatic urethane methacrylate UEDMA, 2 (15%) reacted positively. None of the sensitized guinea pigs in the three different experimental groups reacted when challenged with the other two products (Table 2). None of the control animals reacted positively and all animals were negative to hydroquinone.

DISCUSSION

In the coating industry a high curing speed of the polymers is desirable. Polymers with acrylic unsaturation have higher UV-reactivity than those with methacrylic unsaturation, and thus, they polymerize faster (6).

The acrylated urethanes are not only used as prepolymer in UV-curable coatings but also as resins for dental applications. The acrylated urethanes used in dentistry are mostly of the methacrylated types. One methacrylic resin which is finding significant use in dental composites and sealant applications is urethane methacrylate. This is primarily the alkyl methacrylate resulting from the reaction of such compounds as hydroxy propyl methacrylate or hydroxyethyl methacrylate with an appropriate diisocyanate (10). The two methacrylate groups are thus connected by relatively large, bulky aliphatic or aromatic moieties which also contain two urethane groups. The urethane methacrylate plays the same role as

the more common BIS-GMA (the addition reaction product of bisphenol A and glycidyl methacrylate or an epoxy resin and methacrylic acid) in composite and sealant formulations (11).

The most common of the urethane methacrylates used in dental resins, are of the aliphatic types which is an urethane from bis-ethylene glycol methacrylate (12). The urethane methacrylate (UEDMA) used in this investigation is believed to be of that type with the proposed general structure shown in Fig. 1. The R-group between the two urethane moities probably has the imperical formula $C_{10}H_{20}$. The structure for the R-group is ambiguous, since the aliphatic chain is probably a mixture of several isomers.

Few reports about contact allergy from urethane (meth)acrylates have been published. Dempsey (13) reported 3 cases of contact hypersensitivity to Sta-Lok and Loctite anaerobic sealants. The investigation showed that the antigenic substance in Loctite was a substance labeled "polyurethane dimethacrylate" by the company. Of the 3 patients tested all reacted to "polyurethane dimethacrylate" but also to glycidyl methacrylate, a component in Sta-Lok anaerobic sealant.

Netheercott et al (14) describe seven workers exposed to ultraviolet printing inks which developed contact dermatitis. Six cases were allergic and one irritant. An urethane acrylate resin accounted for five cases of sensitization, one of which was also sensitive to pentaerythritol triacrylate and another to an epoxy acrylate resin. GPM-test was performed with the urethane acrylate. For the highest intradermal induction concentration used, all of the 10 animals became sensitized. The urethane acrylate was believed to be of the aromatic type based on toluene diisocyanate (TDI).

Of the two commercial urethane acrylates tested in this investigation, the aliphatic one (Ebecryl 270) seems to be a more potent sensitizer than the aromatic one (Ebecryl 220).

The aromatic Ebecryl 220 is based on TDI and has a molecular weight of 1000. The aliphatic Ebecryl 270 has a higher molecular weight (1500). No information was given about the type of aliphatic isocyanate used to synthesize Ebecryl 270, but 1,6-hexamethylene diisocyanate (HDI) might be a possible isocyanate moiety. Ebecryl 220 bears 6 functional groups, while Ebecryl 270 only 2. See Fig. 2.

If the number of functional groups attached to the oligomer are of importance for the allergenicity of the compounds are unknown.

The urethane acrylates were analysed on High Performance Liquid Chromatography (HPLC) and the chromatogram showed many peaks for both products suggesting impurities.

Most haptens have a molecular weight below 400. Oligomers with increasing molecular weight have a decreasing sensitizing capacity and it is unlikely that a hapten will have a molecular weight above 1000 (15). Therefore it is probable that any impurities left unreacted, formed or added during the synthesis of the final product might possess allergenic potentials.

Which or what kind of impurity in the product that might be responsible for the allergenicity is impossible to know without further purification and tests as have been shown in previous study (9). If an aliphatic urethane acrylate in general is a stronger sensitizer than the aromatic one is thus unpredictable without further investigations.

The aliphatic urethane methacrylate (UEDMA) is according to the manufacturer 98% pure. This is also in agreement with analysis performed on HPLC. The molecular weight is 470 and bear 2 functional groups. Thus UEDMA itself seems to possess the allergenic potential. Of the guinea pigs tested 15% became sensitized. Thus, UEDMA, is according to Magnusson & Kligman (8) a mild sensitizer (grade II). The aliphatic urethane methacrylate (UEDMA) has a lower allergenicity than corresponding aliphatic urethane acrylate (Ebecryl 270). However, if the statement can be generalized to all aliphatic or aromatic urethane acrylates, that by adding a methyl group at the α -carbon atom in the acrylic moieties decrease the sensitizing potential demand further investigations.

During a period of a year, 702 patients with suspected contact allergy to dental materials have been tested in various Dermatology departments in Sweden with the "Dental Screening Tray" (16). Two of the patients reacted to UEDMA. The relevance of the test results in those two patients is not known (17).

There are many different types of urethane (meth)acrylates in use and still being synthesized both for industrial purposes and for use in dentistry. Which

type of urethane (meth)acrylates to be chosen for the different applications by the consumer is largely dependent on its technical properties. It would be wise both for the manufacturers as well as for the consumers to choose a product not only for its good technical properties but also one with a low sensitizing capacity, to prevent unexpected skin problems among exposed workers.

ACKNOWLEDGEMENTS

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Table 1.

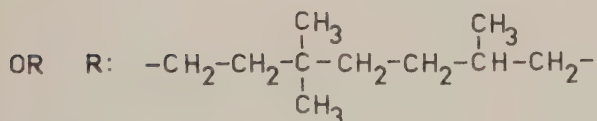
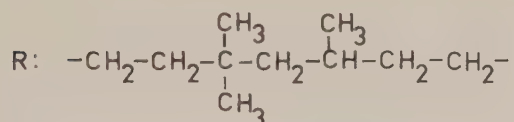
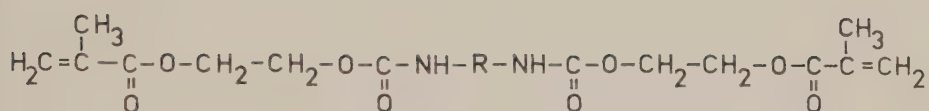
Concentration used for induction and challenge.

Chemical	Trade name	Molecular weight	Purity %	Intradermal concentration in olive oil/acetone (10/1) % w/w	SLS 10% w/w in pet.	Topical concentration % w/w	Challenge concentration in petrolatum % w/w
Urethane acrylate (Aromatic)	Ebecryl 220 (UCB)	1000	Commercial product	1	No	100	0.2
Urethane acrylate (Aliphatic)	Ebecryl 270 (UCB)	1500	Commercial product	1	Yes	100	1
Urethane methacrylate (Aliphatic)	UEDMA (Ivoclar)	470	98	5	Yes	100	1

Table 2.

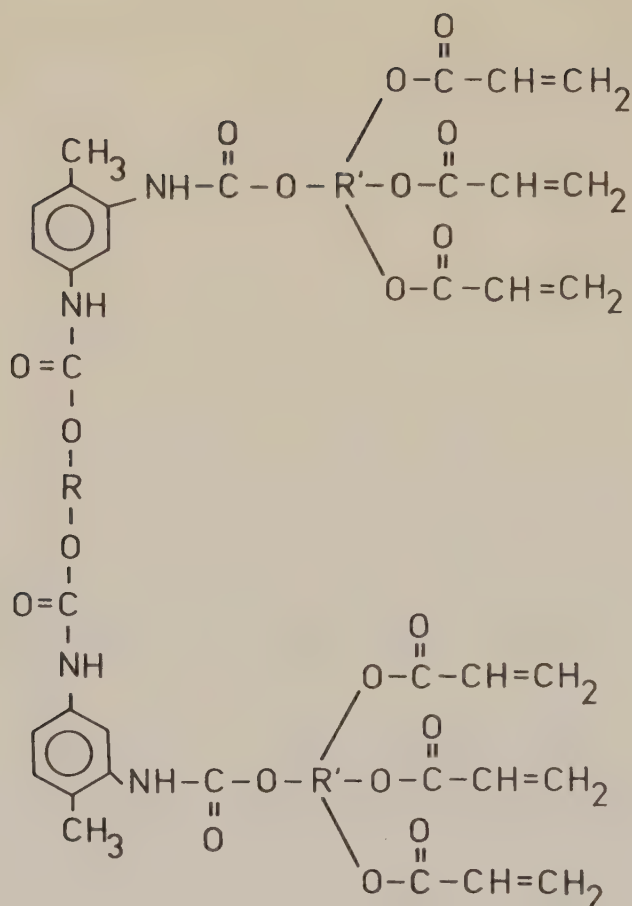
Test results and simultaneous reaction pattern for the different urethane (meth)acrylates.
The number indicate number of positive animals of 15 tested.

Challenged with Sensitized to	Ebecryl 220 (Aromatic)	Ebecryl 270 (Aliphatic)	LEDMA (Aliphatic)
Ebecryl 220 (Aromatic)	2	0	0
Ebecryl 270 (Aliphatic)	0	13	0
LEDMA (Aliphatic)	0	0	2



AND MANY OTHER POSSIBILITIES

Fig. 1. The proposed formula of urethane methacrylate (UEDMA) and its isomers.



EBECRYL 220

Fig. 2. The proposed formula of Ebecryl 220 where R might be an ethylene group and R' a substituted pentaerythritol.

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VIII

THE SENSITIZING CAPACITY OF MULTIFUNCTIONAL
ACRYLATES IN THE GUINEA PIG

Bert Björkner

THE SENSITIZING CAPACITY OF MULTIFUNCTIONAL ACRYLATES IN THE GUINEA PIG

Bert Björkner

Section of Occupational Dermatology, Department of Dermatology, General Hospital, Malmö and Department of Experimental Research General Hospital, Malmö, Sweden. Contact Dermatitis (Submitted for publication)

The multifunctional acrylates used in ultraviolet (UV) curable resins acts as cross-linkers and "dilutents". They are usually based on di(meth)acrylate esters of di-alcohols or tri- and tetraacrylate esters of polyalcohols. In UV-curable coatings the most commonly used are pentaerythritol triacrylate (PETA), trimethylolpropane triacrylate (TMPTA) and 1,6-hexanediol diacrylate (HDDA). In other areas, such as in dental composite resin materials, the dimethacrylic monomers based on n-ethylene glycol are the most useful. The sensitizing capacity of various multifunctional acrylates and their simultaneous reaction pattern have been investigated with the "Guinea Pig Maximization Test" (GPM-test). The tests show that BUDA, (1,4-butanediol diacrylate) and HDDA are moderate to strong sensitizers and that they probably cross-react with each other. The n-ethylene glycol diacrylates and methacrylates tested are weak or non-sensitizers. Tripropylene glycol diacrylate (TPGDA) is a moderate and neopentyl glycol diacrylate (NPGDA) a strong sensitizer, whereas neopentyl glycol dimethacrylate is a non-sensitizer. The commercial PETA is a mixture of pentaerythritol tri- and tetraacrylate (PETA-3 and PETA-4). PETA-3 is a much stronger sensitizer than PETA-4. Simultaneous reactions were seen between PETA-3, PETA-4 and TMPTA. The oligotriacrylate OTA 480 is a moderate sensitizer, but no concomitant reactions were seen with PETA-3, PETA-4 and TMPTA. Of the multifunctional acrylates tested, the di- and triacrylic compounds should be regarded as potent sensitizers. The methacrylated multifunctional acrylic compounds are weak or non-sensitizers.

Key words: Guinea Pig Mazimization Test - multifunctional acrylate - sensitizing capacity.

The three basic components of an ultraviolet (UV) curable resin are: an acrylated prepolymer, a multifunctional acrylate ester and a photoinitiator system. The multifunctional acrylates have a dual role as they serve as cross-linking agents as well as reactive diluents. The multifunctional acrylates are for example di(meth)acrylate esters of dialcohols or tri- and tetraacrylate esters or polyalcohols. Apart from being used in formulations for UV-curable inks or electron beam irradiation curable coatings, the multifunctional acrylates are important acrylic compounds in photopolymer and flexographic printing plates (1 - 3) and photoresists (an etch resist for printed circuit boards) (4). Other areas where the properties of the multifunctional acrylate esters are applicable are in acrylic glues, adhesives and anaerobic sealants. Instead of using methyl methacrylate monomer, the manufacturers of artificial nail preparations, have changed their formulations and are now using methacrylate ester monomers. Some of the more commonly used are ethylene glycol dimethacrylate (EGDMA), trimethylolpropane trimethacrylate (TMPTMA) and diethylene glycol dimethacrylate (DEGDMA) (5).

Many dental composite resin materials and denture base polymers are "diluted" with "difunctional" acrylates. These are dimethacrylic monomers, of which ethylene glycol dimethacrylate (EGDMA), diethylene glycol dimethacrylate (DEGDMA), triethylene glycol dimethacrylate (TREGDMA) and 1,4-butanediol dimethacrylate (BUDMA) are used most extensively (6 - 10).

The purpose of this study was: 1) to investigate the sensitizing capacity of the most commonly used multifunctional acrylates with the "Guinea Pig Maximization Test" (11, 12), 2) to investigate their simultaneous reaction pattern with other structurally related acrylic compounds and other substances of interest.

MATERIAL AND METHODS

CHEMICALS

The following acrylic compounds were tested in this investigation:

Diacrylates. 1,4-Butanediol diacrylate (BUDA), 1,6-hexanediol diacrylate (HDDA)*, diethylene glycol diacrylate (DEGDA), tetraethylene glycol diacrylate (TEGDA)**, tripropylene glycol diacrylate (TPGDA) and neopentyl glycol diacrylate (NPGDA)

were all purchased from UCB, Belgium. Triethylene glycol diacrylate (TREGDA)** was purchased from Sartomer Comp., West Chester, USA. Ethylene glycol diacrylate (EGDA) was not available.

Dimethacrylates. 1,4-Butanediol dimethacrylate (BUDMA) was obtained from Rohm & Haas Comp., USA, 1,6-hexanediol dimethacrylate (HDDMA) from Sartomer Comp., West Chester, USA, ethylene glycol dimethacrylate (EGDMA) from Fluka AG, Switzerland, triethylene glycol dimethacrylate (TREGDMA) from Koch-Light Laboratories Ltd, England, tetraethylene glycol dimethacrylate (TEGDMA) and neopentyl glycol dimethacrylate (NPGDMA) from Sartomer Comp., West Chester USA. Diethylene glycol dimethacrylate (DEGDMA) and tripropylene glycol dimethacrylate (TPGDMA) were not available.

Tri- and tetraacrylates. Oligotriacrylate 480 (OTA 480) was purchased from UCB, Belgium. Pentaerythritol triacrylate (PETA-3) and pentaerythritol tetraacrylate (PETA-4) were obtained from Alfa Products, Thiokol/Ventron Div., USA.

The structural formulae of the acrylic compounds tested in this investigation are shown in Fig. I - III.

Other chemicals used in this investigation were pentaerythritol purchased from Akzo Chemie, The Netherlands, trimethylolpropane and hydroquinone, both from Fluka AG, Switzerland. The Freund's Complete Adjuvant (CFA) was purchased from Difco Laboratories, USA.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY - ANALYSIS

The purity of all acrylic compounds was analysed by means of High Performance Liquid Chromatography (HPLC). An isocratic system was used (Laboratory Data Control, Milton Roy Company, USA) comprising a Constametric III pump and a

* HDDA is also called HDODA by some authors.

** In the literature TEGDA is commonly used as an abbreviation for triethylene glycol diacrylate instead of TREGDA. In this study TEGDA is used to abbreviate tetraethylene glycol diacrylate, which hopefully will be less confusing.

variable UV-detector, Spectro Monitor III. The same additional equipment as described in a previous study (13) was used. For the di(meth)acrylates a Nucleosil C-18, 5 μ (1200 mm $\times$ 4.6 mm i.d.) analytical reversed phase column was used (Macherey-Nagel & Co., Düren) and for the purification and isolation of PETA-3 and PETA-4, a Partisil M9 C-18 (100 mm $\times$ 9.4 mm i.d.) semi-preparative reversed phase column was used (Whatman, USA). In order to check the purity of the collected fractions of PETA-3 and PETA-4 an analytical Spherisorb C-18, 5 μ reversed phase column was used.

The following chromatographic conditions are given:

	<u>Nucleosil C-18</u>	<u>Partisil M9 C-18</u>	<u>Spherisorb C-18</u>
Mobile phase	CH ₃ CN:H ₂ O (80/20)	CH ₃ CN:H ₂ O (70/30)	CH ₃ OH:H ₂ O (53/47)
Flow rate	1 ml/min	1.7 ml/min	1 ml/min
Detection	UV at 235 nm	UV at 220 nm	UV at 220 nm

THE GUINEA PIG MAXIMIZATION TEST (GPM-TEST)

The GPM-test was performed in accordance with the original description (11,12) and under the same conditions as in previous studies (13). Approximately 15 female albino guinea pigs of the Dunkin-Hartley strain, weighing 300-400 grams, were used as experimental animals and the same number as control animals for each acrylate tested.

Induction procedure. For each acrylic compound three animals were used to evaluate the optimal concentration for intradermal and topical induction. These animals were not subsequently used in the sensitization experiments. To get a better dispersion for intradermal injection, the test substance were dissolved in a mixture of olive oil and acetone (9 parts olive oil to 1 part acetone). Only for the dimethacrylates tested, pretreatment with 10% sodium lauryl sulphate (SLS) in petrolatum before the topical induction was necessary. Induction was performed with the commercial acrylic products. The final concentrations for intradermal and topical induction are shown in Table 1.

Challenge procedure. The challenge concentrations had previously been evaluated on 4 unused animals for each chemical tested. Petrolatum was used as vehicle for all test substances. The amount of test material was approximately 0.015 g.

In order to investigate cross-sensitivity or concomitant reactions, the animals apart from being challenged with the product used for induction, were also tested with similar or structurally related acrylic compounds. Because quite a few of the acrylates have similar chemical structures and therefore might be of interest for simultaneous reaction studies, the animals were challenged twice, one week apart, with a maximum of six test sites on the flank each time. 48 h. after the first challenge application the animals received a booster dose of the sensitizing chemical applied intradermally on the neck in the same concentration and vehicle used for the intradermal induction procedure, but without (CFA) (14). The acrylate used for induction was also retested in the second challenge procedure. The test response was read and evaluated in the same manner as described earlier (13). If no animals or just a few animals showed positive reactions, the GPM-test procedure was repeated once more. All di(meth)acrylates and OTA 480 were tested using the commercial products. Animals sensitized with PETA-3 and PETA-4 were tested with the commercial products and the main fraction of the compounds. In all animals, challenge with hydroquinone (0.1% w/w in alcohol) was performed.

The control animals were sensitized in the same way as the experimental group but without the acrylic compounds. When the sensitized animals were challenged, the control animals were also patch tested with the same chemicals and the same concentrations. As a booster dose, the control animals received olive oil intradermally.

RESULTS

HPLC-ANALYSIS

All the acrylic compounds used for induction and challenge were commercial products. Most of the di(meth)acrylates had, however, a purity of at least 95% (some 98%). Only HDDA, TPGDA and NPGDA were considered to be impure as, besides the main peak, many small peaks were seen in the chromatograms. OTA 480 had a purity of 95% and PETA-3 contained a high degree of impurities. One peak in the HPLC-chromatogram was identified as PETA-4. As the PETA-3 compound polymerized very easily, complete purification was impossible. The final "purified" product of PETA-3 contained approximately 1% of PETA-4. The commercial PETA-4 was more pure than PETA-3 and the HPLC-chromatogram of the "purified" PETA-4 showed mainly two peaks. The smaller peak (approx-

mately 15% of the main peak) was identified as PETA-3. As the PETA compound is a mixture of pentaerythritol tri- and tetraacrylate further purification was impossible.

GPM-TEST

Diacrylates. Of the 15 animals sensitized to BUDA, 10 (67%) showed positive test reactions. Nine (60%) of the 15 animals sensitized with HDDA were positive. Two of the 15 animals (13%), sensitized with DEGDA reacted positively. None of the animals sensitized with TREGDA and TEGDA showed any reaction. Of the guinea pigs sensitized with TPGDA, 11 of 15 (73%) became allergic. All of the 14 animals sensitized to NPGDA gave a positive test response.

Dimethacrylates. Only 1 animal of 15 (7%) became sensitized to TREGDMA. No sensitization was observed for any of the other dimethacrylates tested (BUDMA, HDDMA, EGDMA, TEGDMA and NPGDMA).

The simultaneous reaction pattern for the di(meth)acrylates tested is shown in Table 2. Of the 10 animals sensitized to BUDA, 7 reacted also to HDDA, 3 to DEGDA and 1 to TPGDA. Challenge with TEGDA and NPGDA were negative. Of the 9 guinea pigs sensitized to HDDA, 1 reacted to BUDA, 4 to DEGDA and TEGDA. No animals reacted positively to TPGDA and NPGDA. Some of the 11 TPGDA-sensitized animals reacted also to BUDA (7), DEGDA (10) TEGDA (10) and NPGDA (4) but none to HDDA. All of the 14 animals became sensitized to NPGDA and 8 of those reacted to BUDA, 11 to DEGDA, 10 to TEGDA and 3 to TPGDA but none to HDDA. None of the animals sensitized to a diacrylate reacted to any dimethacrylate. Only one animal became sensitized to - TREGDMA. That animal did not react to any of the other di(meth)acrylates tested.

Tri- and tetraacrylates. Ten (67%) of 15 guinea pigs became sensitized to commercial PETA-3 and 6 of those also reacted to "purified" PETA-3, 7 to commercial PETA-4, 3 to "purified" PETA-4, 7 to TMPTA and 3 to pentaerythritol. No reactions were observed when the animals were challenged with OTA 480.

Of the 15 guinea pigs sensitized with OTA 480, 6 (40%) were positive. None of these reacted when challenged with PETA-3, PETA-4, TMPTA or trimethylol-propane.

Only 1 animal (7%) of the 15 sensitized with commercial PETA-4, reacted positively. The same animal reacted also to "purified" PETA-4 and PETA-3 and to TMPTA. However, 2 other animals, which did not give any test response to PETA-4 reacted to PETA-3 only in its commercial form. No animals reacted when challenged with OTA 480 or pentaerythritol. See Table 3.

Challenge with hydroquinone gave no response in any of the animals tested.

None of the control animals reacted positively to any of the acrylic compounds tested.

DISCUSSION

The present investigation demonstrates that HDDA and BUDA are moderate to strong sensitizers (grade III - IV) according to classification by Magnusson & Kligman (11). In a study made by van der Walle et al (15), it was found that the sensitizing potential of BUDA was questionable, as the intensity of the test reactions decreased in successive challenges. They also failed to demonstrate a sensitizing potential of HDDA in a GPM-test. However, HDDA appeared to be a sensitizer in a Freund's Complete Adjuvant Test (FCAT).

Malten et al (16) observed an epidemic of a peculiar type of delayed irritant contact dermatitis from HDDA and BUDA in an electron beam coating department of a door factory. Contact sensitization to the chemicals was not observed. According to Malten et al (16) Nethercott sensitized 4 of 10 guinea pigs to HDDA using the maximization test (17).

It is questionable, if BUDA and HDDA then cross-react. Of the 9 animals sensitized with HDDA, only 1 reacted to BUDA, but of the 10 animals sensitized with BUDA, 7 reacted also to HDDA. HDDA is 85% pure and BUDA has a purity of 98%. HPLC-analysis showed that HDDA did not contained BUDA and BUDA did not contained HDDA. However, simultaneous reaction between BUDA

and some structurally related impurity in HDDA, can not be excluded. No reactions were seen with any of the di(meth)acrylates tested.

van der Walle & Bensink (18) found that one animal sensitized to HDDA reacted clearly to HDDMA. The same animal, however, showed positive test reactions even to all the other acrylates tested.

Parker & Turk (19) succeeded in sensitizing guinea pigs to HDDA as well as to TRPGDA, TMPTA and PETA and some other acrylates, using the Polak method of immunisation. However, they were unable to induce contact sensitivity to acrylic compounds that had a methyl group on the α -carbon atom (carbon 3) in the acrylic moiety (TREGDMA, EGDMA, TMPTMA and other methacrylates).

This is in agreement with the results found in this investigation where most of the dimethacrylates tested were non-sensitizers. Only 1 animal of 15 reacted to TREGDMA in this study. The same result was given in a repeated GPM-test.

TREGDA as well as TEGDA are non-sensitizers according to this investigation.

Our findings do not confirm the results from Cavelier et al (20), who studied the sensitizing potential of n-ethylene glycol diacrylates and dimethacrylates in the GPM-test. They found TREGDA and TEGDA to be moderate sensitizers (grade III) and they also observed cross-reaction between the two diacrylates. Cavelier et al (20) also found that the dimethacrylates EGDMA, DEGDMA, TREGDMA and TEGDMA were sensitizers. This is not in agreement with the findings in this study or from the results achieved by Parker & Turk (19).

Patients with suspected contact allergy to dental materials have been patch tested in various dermatology departments in Sweden during one year, with the "Dental Screening Tray" (21). Of the 702 patients tested, 5 reacted positively to EGDMA and 2 to TREGDMA. The relevance of the test results is uncertain (22).

Malten & Bende (2) described 5 workers who developed an allergic contact dermatitis while working with a photoprepolymer printing plate procedure. Two of 4 patients who were patch tested with dilutions of the ingredients showed positive patch test reactions to DEGDMA and TEGDMA. In order to obtain information about possible cross-reaction, one of the patients was also tested

with EGDMA, HDDA and some other acrylic compounds. The patient reacted to the EGDMA solution only. The authors emphasize that the substances tested were of industrial quality.

Beurey et al (23) observed bullous irritant reactions in workers in a large newspaper plant manufacturing printing plates using photopolymer acrylic resins in place of lead. The irritant reactions were ascribed to the irritant effect of TEGDA. Experimentally, EGDA and TREGDA were even more irritant at the same concentrations. Replacement of these diacrylates by dimethacrylates solved the irritancy problem. In addition, the authors described four service men who appeared to have a true allergic contact dermatitis because they reacted to patch tests with TEGDA. Its replacement by TEGDMA eliminated the problem.

Whittington (24) reports that an employee developed a dermatitis while working on the formulation of a pressure sensitive adhesive for sanitary napkins which contained UV-curable monomer and multifunctional acrylates. Patch tests to work contacts showed sensitivity to TEGDA. The patient was also patch test positive to HDDA but negative to PETA, TMPTA and methyl methacrylate. HDDA was a non workrelated acrylate.

TPGDA and NPGDA tested in this investigation, were found to be strong to extreme sensitizers. However, both acrylates are impure and thus, it is uncertain if the proper allergens are the acrylic monomers or its impurities.

The most commonly used multifunctional acrylate in an UV-curable ink or coating formulation, is an acrylic acid ester of either pentaerythritol (PETA), trimethylolpropane (TMPTA) or hexanediol (HDDA). Film flexibility, hardness, chemical resistance and viscosity are coating properties that can be controlled by the proper selection of the multifunctional acrylate.

PETA has the highest degree of acrylation (on the average 3.3 acrylate groups per mole) and provides the highest degree of cross-linked density. PETA has found application in a variety of UV-cure applications and is especially favored for UV-cured letterpress and flexographic inks (25).

TMPTA incorporates most of PETA's desirable features in addition to having a low viscosity. HDDA has the lowest viscosity of the three multifunctional

acrylates. It might be expected to find application in such areas as UV-curable gravure inks, and sprayable coatings (25). The trifunctional acrylates are unacceptable on metallic surfaces, while difunctional diluents, such as HDDA, markedly improve the adhesion of the coatings (26).

The commercially available PETA is a mixture of pentaerythritol tri- and tetraacrylates. The PETA-3 used in this investigation consisted according to the manufacturer, mostly of pentaerythritol triacrylate. HPLC-analysis showed that the compound contained a high degree of impurities. One fraction was identified as pentaerythritol tetraacrylate. Complete purification of PETA-3 was impossible as the compound polymerized very easily. The "purified" PETA-3 contained approximately 1% of PETA-4. PETA-4 had a higher degree of purity, and the HPLC chromatogram showed mainly two peaks. The smaller peak (approximately 15% of the main peak) was identified as PETA-3.

PETA-3 seems to be a much stronger sensitizer than PETA-4 (Table 3). Simultaneous reactions were also seen between PETA-3 and PETA-4. Some PETA3 sensitized animals also reacted to pentaerythritol. If that substance is the proper allergen in PETA-3 is not known. Those animals sensitized to PETA3 and PETA-4 also reacted to TMPTA. This support the results found in a previous study of probable cross-reactivity between PETA and TMPTA (27).

The fact that PETA is a potent sensitizer has also been reported by other investigators. Nethercott (28) found PETA to possess a high potential to induce cutaneous allergy in the GPM-test. It was confirmed in repeated experiments (29, 30). The challenge concentration used in their studies was 10% in petrolatum, which we found to be irritant.

Parker & Turk (19) found PETA to be a sensitizer using the Polak method of immunization.

Contact allergy to PETA has also been described in humans. Emmett & Kominsky (31) report that eight men employed in the manufacture of UV-cured inks developed allergic contact dermatitis predominantly on the exposed areas. Patch tests revealed sensitization to PETA in 4 employees, TMPTA in 7 employees, to HDDA in 6 employers and to epoxy acrylate oligomers in 3 of the 8 employees. The authors suggest that it is possible that some of the observed reactions represent cross-reactions but as the employees were potentially

exposed to each of the materials this possibility can not be evaluated in their study.

Investigation of a dermatitis incident involving the handling of UV-type ink by two press minders is described by Smith (32). Patch testing showed positive reactions to 2 of the inks used but not to the third. The 2 workers were also positive to PETA, one of the acrylic components in the UV-ink. The 2 printers were also positive to TMPTA and tripropylene glycol triacrylate which was not a constituent in the inks used. The author suggests possible cross-sensitization reactions between PETA and TMPTA vs. tripropylene glycol triacrylate. (According to Malten (33), the last-named substance does not exist: possibly Smith meant tripropylene glycol diacrylate, TRPGDA).

Emmet (34) reported that 5 out of 26 employees developed eczematous dermatitis while formulation radiation drying printing inks. Positive patch tests were obtained with PETA and TMPTA and with the inks containing these in 4 affected employees. Cross-sensitization was observed to an acrylic compound to which the workers had not been exposed, dipentaerythritol monohydroxy pentaacrylate in each case. As all subjects had had potential exposure to PETA and TMPTA, the author could not determine whether the multiple reactivity was a result of cross-sensitization or concomitant sensitization. The fifth employee appeared to have developed irritant dermatitis from contact with these materials. The author stresses the fact that one of the commercial acrylic monomers consist of pure compounds, but no effort was made to study the many potential contaminants present in what were obviously mixtures.

Fifteen cases of contact dermatitis and 3 of irritant contact conjunctivitis, due to multifunctional acrylic monomers in printing inks in an ink manufacturing plant are described by Nethercott (29). Seven with dermatitis were sensitized to PETA, 2 reacted to TMPTA and none to HDDA and methyl methacrylate. In an experiment performed on human volunteers, Nethercott (29) was able to sensitize 7 of 9 to PETA.

Whittington (35) had a patient who was formulating a UV-resin based vehicle and developed hypopigmentation on the forearms without preceding dermatitis. A non-resin additive, quinone, alone or in conjunction with a likely contaminant, hydroquinone, was the suspected agent. The patient had positive patch test

reactions to the UV-resin vehicle as well as to PETA, alone and in mixtures. The reaction to PETA was not pertinent to clinical findings.

Dahlquist et al (36) describes 4 workers who developed hand and face dermatitis when exposed to a floor top coat. This contained a polyurethane and a poly-functional aziridine hardener and additives. The aziridine hardener was made by reacting propyleneimine with TMPTA. All 4 reacted to the hardener and to TMPTA which was present in excess. Two of them also reacted to PETA. PETA can be used in the production of aziridine hardeners instead of TMPTA. The authors stress the fact that well-known sensitizers can be found in hidden sources when used in a quite different chemical process.

OTA 480 is a moderate sensitizer (grade III) according to this investigation. OTA 480 is an oligotriacrylate with a molecular weight of 480. The manufacturer did not agree to reveal the chemical formulae, but OTA 480 was said to be used as a substitute for other triacrylates such as TMPTA and PETA. To my knowledge, no cases of contact allergy to OTA 480 have yet been reported.

When the sensitizing capacity of chemicals are investigated it must be stated if the compounds are pure or of industrial quality. The purity is even more important when cross-reactivity between two chemicals is discussed. An impurity in the substance under test might be an allergen, and thus lead to faulty interpretation of the test result. In this investigation the acrylic compounds tested were of sufficient purity, except for HDDA, TPGDA, NPGDA OTA 480 and the PETA compounds discussed previously.

This investigation thus clearly demonstrates that of the multifunctional acrylic monomers commonly used on the market, the di- and triacrylic compounds should, with some exceptions, be regarded as relatively potent sensitizers. The methacrylated multifunctional acrylates are weak or non sensitizers. It might therefore be wise when formulating an UV-curable ink or coating, to choose a methacrylated compound with high purity in the composition in order to minimize the risks for contact sensitization among the exposed workers.

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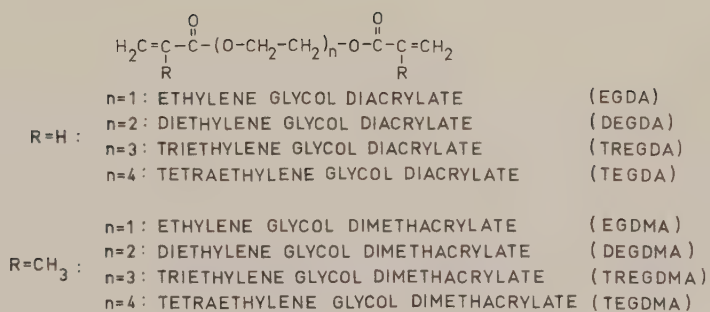


Fig. 1. Chemical structure of n-ethylene glycol di(meth)acrylates.

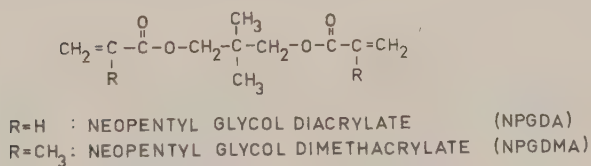
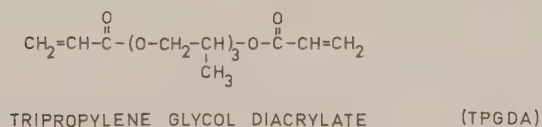
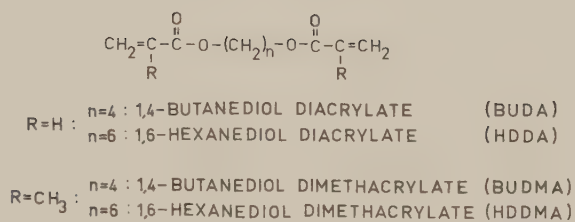
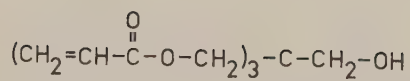
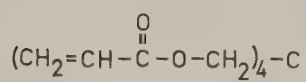


Fig. 2. Structural formulae of di(meth)acrylates based on butanediol, hexanediol, tripropylene glycol and neopentyl glycol.



PENTAERYTHRITOL TRIACRYLATE

(PETA-3)



PENTAERYTHRITOL TETRAACRYLATE

(PETA-4)

Fig. 3. Structural formulae of pentaerythritol tri- and tetraacrylate.

Table 1. CONCENTRATIONS USED FOR INDUCTION AND CHALLENGE FOR THE ACRYLIC COMPOUNDS TESTED IN THIS INVESTIGATION

pet. = petrolatum,	ac. = acetone,	- = SLS not used,	+ = SLS used			
Chemical	Abbreviation	Molecular weight	Intradermal concentration in olive oil/ac. (9:1) % w/w	SLS 10% w/w in pet.	Topical induction concentration in pet.	Challenge concentration in pet. % w/w
-diacrylate						
1,4-butanediol	BUDA	198	1	-	25	0.2
1,6-hexanediol	HDDA	226	1	-	25	0.2
diethylene glycol	DEGDA	214	1	-	25	0.2
triethylene glycol	TREGDA	258	1	-	25	0.2
tetraethylene glycol	TEGDA	302	1	-	25	0.2
tripropylene glycol	TPGDA	300	1	-	25	0.2
neopentyl glycol	NPGDA	212	1	-	25	0.2
-dimethacrylate						
1,4-butanediol	BUDMA	226	2	+	50	1.0
1,6-hexanediol	HDDMA	254	5	+	50	1.0
ethylene glycol	EGDMA	198	5	+	50	1.0
triethylene glycol	TREGDMA	286	1	+	50	1.0
tetraethylene glycol	TEGDMA	330	5	+	50	1.0
neopentyl glycol	NPGDMA	240	5	+	50	1.0
-triacrylate						
pentaerythritol	PETA-3	298	1	-	25	0.2
oligo	OTA-480	480	1	-	25	1.0
-tetraacrylate						
pentaerythritol	PETA-4	352	1	-	25	0.2

Table 2. CHALLENGE REACTIONS AND SIMULTANEOUS REACTION PATTERN IN ANIMALS SENSITIZED WITH DI(METH)-ACRYLATES.

The figures indicate the positive animals of the animals tested. - = not tested.

Challenged with	Number of animals tested	DIACRYLATE						DI(METH)ACRYLATE					
		BUDA	HDDA	DEGDA	TREGDA	TEGDA	TPGDA	NPGDA	BUDMA	HDDMA	EGDMA	TREGDMA	NPGDMA
<u>Diacrylate</u>													
BUDA	15	10	7	3	-	0	1	0	0	-	0	-	-
HDDA	15	1	9	4	-	4	0	0	0	0	-	-	-
DEGDA	15	3	0	2	-	3	0	0	-	-	0	0	-
TREGDA	19	-	-	0	0	0	0	-	-	-	-	0	-
TEGDA	15	0	0	0	-	0	-	0	-	-	-	0	-
TPGDA	14	7	0	10	-	10	11	4	-	-	0	0	-
NPGDA	14	8	0	11	-	10	3	14	-	-	0	0	0
<u>Di(meth)acrylate</u>													
BUDMA	10	0	0	-	-	0	-	-	0	-	0	-	-
HDDMA	15	0	0	0	-	0	0	-	0	0	-	0	-
EGDMA	13	0	0	-	-	0	-	-	0	-	0	0	-
TREGDMA	15	0	0	-	0	-	-	-	0	-	0	1	-
TEGDMA	15	-	-	-	-	0	-	-	-	-	0	0	-
NPGDMA	15	-	-	-	-	0	-	0	-	-	-	0	0

Table 3. CHALLENGE REACTIONS AND SIMULTANEOUS REACTION PATTERN IN ANIMALS SENSITIZED WITH TRI- AND TETRAACRYLATES

The number indicate the positive animals of 15 tested.

- = not tested

Challenged with	PETA-3	PETA-3 MAIN FRACTION	OTA 480	PETA-4	PETA-4 MAIN FRACTION	TMPTA	PENTA- ERYTHRITOL	TRI- METHYLOL- PROPANE
Sensitized to								
PETA-3	10	6	0	7	3	7	3	-
OTA 480	0	-	6	0	-	0	-	0
PETA-4	3	1	0	1	1	1	0	-

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IX

INFLUENCE OF THE VEHICLE ON ELICITATION OF
CONTACT ALLERGIC REACTIONS TO ACRYLIC COMPOUNDS
IN THE GUINEA PIG

Bert Björkner and Bo Niklasson

INFLUENCE OF THE VEHICLE ON ELICITATION OF
CONTACT ALLERGIC REACTIONS TO ACRYLIC COMPOUNDS
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Bert Björkner and Bo Niklasson

Section of Occupational Dermatology, Department of Dermatology, General Hospital, Malmö and Department of Experimental Research General Hospital, Malmö, Sweden. Contact Dermatitis (Submitted for publication)

Many factors can influence the elicitation of hypersensitivity reactions in guinea pigs and human beings. The effect the vehicle might have on the test response in guinea pigs sensitized with various acrylic compounds, using the "Guinea Pig Maximization Test" (GPM-test), has been investigated. A marked decrease in the number of positive animals was seen when acetone was used as test vehicle, compared to petrolatum. The same result was seen with alcohol as vehicle, when neopentyl glycol diacrylate (NPGDA) was used as an acrylic monomer model. The patch test locations on the guinea pig flank, also effected the test response. Half of the animals did not react when challenged near the abdomen, compared to a test site near the back. By means of HPLC-analysis, a possible adsorption of the acrylic monomer to the aluminium chamber or filterpaper disc, was estimated. Our findings did not indicate that adsorption occur. A decrease in the amount of acrylic monomer in the test solutions with increasing time, was noted. There was a marked difference in the monomer residue between solutions with (darkness) and without (daylight) inhibitor. The monomer decrease was also more effected by an aluminium surface than a glass or a filterpaper surface. Aluminium oxide, probably enhance the polymerization process. The discrepancy between the test results in this study, when petrolatum and acetone was used as test vehicle, is most certainly due to a polymerization process of the acrylic compounds. Thus, the petrolatum vehicle probably prevents the acrylic monomer to polymerize.

Key words: Acetone - Alcohol - Acrylates - Adsorption - Elicitation - Guinea Pig Maximization Test - Neopentyl glycol diacrylate - Petrolatum - Polymerization - Vehicle.

When testing various acrylic compounds using the "Guinea Pig Maximization Test" (GPM-test) (1, 2) in previous investigations (3, 4, 5), we found a marked discrepancy in the challenge response depending upon whether petrolatum or acetone was used as test vehicle. Various factors, that might effect the challenge response in the GPM-test, have been thoroughly investigated by Magnusson & Kligman (2). However, little research has been done on the influence the test vehicle and chemical conditions might have on the elicitation of hypersensitivity.

The aim of the study was:

- I: By means of the GPM-test,
 - a) to compare the challenge response using petrolatum and acetone as test vehicles in guinea pigs sensitized to various acrylic compounds,
 - b) to investigate the influence of various vehicles on the test response in guinea pigs sensitized to neopentyl glycol diacrylate (NPGDA),
 - c) to study possible regional variations in the challenge response with various vehicles in guinea pigs sensitized to NPGDA.
- II. By means of chemical analysis with High Performance Liquid Chromatography (HPLC) using NPGDA as a model for the acrylate monomer,
 - a) to estimate adsorption of the acrylic monomer to the aluminium chamber and/or to the filterpaper disc,
 - b) to study if any polymerization of the monomer (with or without inhibitor) occurs on the aluminium chamber. Special interest is focused on the alteration of the amount of residue monomer with increasing time,
 - c) to study the influence the surfaces on various materials might have on a possible polymerization process,
 - d) to detect a possible decrease of the monomer content with increasing time, when petrolatum is used as vehicle.

MATERIAL AND METHODS

I. THE GPM-TEST

The following acrylic compounds were investigated.

Prepolymers. Epoxy acrylate DOW, Epikote acrylate DRH-370, BIS-PMA, BIS-EMA, BIS-GMA and BIS-MA are di(meth)acrylates based on bisphenol A or epoxy resin.

Their chemistry and sensitizing potential have been thoroughly studied in a previous paper (3). Ebecryl 220, Ebecryl 270 and UEDMA are urethane (meth)acrylates and have also been described in detail (4). Both induction and challenge was performed with the commercial products.

Multifunctional acrylates. Pentaerythritol triacrylate (PETA-3), pentaerythritol tetraacrylate (PETA-4), oligotriacrylate 480 (OTA 480), 1,4-butanediol diacrylate (BUDA), 1,6-hexanediol diacrylate (HDDA), diethylene glycol diacrylate (DEGDA), tetraethylene glycol diacrylate (TEGDA), tripropylene glycol diacrylate (TPGDA) and neopentyl glycol diacrylate (NPGDA) (Fig. 1), have been investigated and discussed in detail previously (5). Both induction and challenge was performed with the commercial products.

I:a. The GPM-test was performed in accordance with the original description (1, 2). The concentrations used for induction and challenge are exactly the same for the various acrylic compounds as in previous investigations (3, 4, 5). Fifteen guinea pigs were used for each acrylate tested. The vehicles used for challenge were petrolatum and acetone. The test concentrations used were the same for both vehicles and the same as in previous investigations (3, 4, 5). The two challenge tests were applied simultaneously on the right flank. The substance dispersed in petrolatum was applied in an approximate amount of 0.015 g in an aluminium chamber (Finn Chamber, Epitest Ltd, OY, Helsinki, Finland) on the test site 2, and the test substance in the acetone vehicle was applied in an amount of 0.02 ml on a filterpaper disc in the aluminium chamber on test site 5 (Fig. 2). The occlusive bandage was applied in the same way as in previous studies and was left in place for 24 h.

I:b. The test vehicle. As NPGDA is an extreme sensitizers, it was used as a model for the acrylic monomers. The induction procedure was the same as in a previous investigation (5). The challenge concentration was 0.2% w/w, and the vehicles were petrolatum, acetone, alcohol (99.5%) and a mixture of petrolatum and acetone (10 parts of acetone mixed with 90 parts petrolatum). The challenge procedure was performed with Finn Chambers and with the possibility of using a maximum of 6 patch test sites on the guinea pig flank (Fig. 2). In the first challenge the test was performed on the right side of the animal. The test substance in petrolatum, in an approximate amount of 0.015 g, was applied in the aluminium chamber on test site 1, without a filterpaper disc. On test site 3, NPGDA in acetone, on test site 4, NPGDA in petrolatum/acetone and on test site 6, NPGDA in alcohol, was applied

The acrylate dissolved in acetone and alcohol was applied in an amount of 0.02 ml on a filterpaper (cellulose) disc in the aluminium Finn Chamber. The animals received 48 h. after the first challenge application, a booster dose with NPGDA intradermally on the neck in the same concentration and vehicle as used for intradermal induction, but without Freund's Complete Adjuvant (CFA). Test sites 2 and 5 were not used in this study.

I.c. The test site location. One week later the animals were shaved and tested on the left flank. The test sites 1, 2 and 3 are also in the second challenge located most caudally. NPGDA (0.2% w/w) in acetone, was applied at test sites 1 and 6, and in petrolatum at test sites 3 and 4 (Fig. 2). The test substances were applied in the same manner as described above, in the same amount, with the acetone vehicle on a cellulose disc and the petrolatum vehicle in the aluminium chamber. Test sites 2 and 5 were not used.

The number of positive animals and the mean response were recorded. The mean response (m.r) is the sum of the numerical readings of the positive challenge responses from the various animals, divided by the total number of readings, including the negatives.

II. CHEMICAL ANALYSIS

All analysis in this study were carried out by means of an isocratic High Performance Liquid Chromatography System (HPLC), comprising a LDC-Constate-metric III pump, fitted with a Rheodyne loop injector (20 μ l) and a variable UV-detector, LDC Spectro Monitor III (Laboratory Data Control, LDC, Milton Roy Company, USA). The column was a 200mm x 4.6mm, i.d., Li-Chroma column packed with Nucleosil C-18 5 μ particles. The chromatographic conditions were the following:

Mobil phase:	Acetonitrile/water (60:40)
Flow rate:	1 ml/min
Pressure:	2000 psi
Detection:	UV at 235 nm

Chemicals. The following chemicals were used in the HPLC-analysis:

Neopentyl glycol diacrylate (UCB, Belgium); Acetonitrile, HPLC-grade (Carlo Erba, Italy); Acetone, 99.7% (Carlo Erba, Italy); Water (deionized & Millipore R filtrated); Hydroquinone (Fluka AG, Switzerland); Finn Chamber (Epitest Ltd, OY, Finland); Petrolatum (Nord P., Sweden); Filterpaper disc (Epitest Ltd, OY, Finland).

Procedure.

II:a Adsorption 4 ml of a 0.01% v/v solution of NPGDA in acetonitrile is incubated for 1 h. with 1 g of filterpaper discs in a closed vial. 1 g of aluminium chambers is treated in the same manner as the discs. 20 μ l of the two solutions is injected into the chromatograph and the height of the main peak is measured. As a reference 20 μ l of a solution of 0.01% v/v NPGDA in acetonitrile, is injected and the main peak height is determined. Three injections of each solution are made and the average heights are estimated.

II:b. Polymerization. Four different solutions of NPGDA (0.2% v/v) are prepared, two of them using acetone as diluent and the other two acetonitrile. To one acetone solution and one acetonitrile solution, the polymerization inhibitor hydroquinone is added to a total concentration of 0.03% w/w. 25 μ l of the solutions without inhibitor is then transferred with a Carlsberg pipette onto the surface of 5 aluminium chambers each who are left in daylight at room temperature. The chambers are then extracted with 250 μ l acetonitrile at 5, 10, 15, 30 and 60 minutes respectively and a subsequent injection of 20 μ l of these extracts into the chromatograph then follows. The same procedure is repeated for the solutions containing hydroquinone, but instead of daylight the chambers with the solution are left in darkness. As a reference, 20 μ l of a 0.02% v/v solution of NPGDA in acetonitrile is injected. The resulting main peak heights of the chromatograms are then estimated.

II:c. Surface. A similar study as described in part II:b, was performed with filterpaper and a flat glass surface as support for the NPGDA solution (not containing hydroquinone). The study was performed in daylight.

II:d. Petrolatum. NPGDA dispersed in petrolatum at a concentration of 0.2% w/w is applied on the surface of 2 separate aluminium chambers and the exact amount of dispersion is determined with an analytical balance. After 2 h. application in daylight, one of the chambers is extracted at 80°C with 1.0 ml of acetone for 3 minutes in a closed vial. After 24 h. the same procedure is repeated with the other chamber. 20 μ l of the extracts are injected into the chromatograph and the height of the resulting main peaks of NPGDA are measured. As a reference, 20 μ l of a 0.02% v/v solution of NPGDA is used.

RESULTS

I. THE GPM-TEST

I:a. The test results for the various acrylic compounds in acetone and petrolatum are shown in Table 1.

I:b. The test vehicle. With petrolatum as vehicle, 17 of the animals were positive, with acetone 7, petrolatum/acetone 90/10) 18 and alcohol 6 of the 20 guinea pigs tested (Table 2).

I:c. The test site location. In the first challenge, with petrolatum as vehicle, 17 of 20 animals were positive (m.r. 1.5) at test site 1. In the second challenge 15 of the 20 animals (m.r. 1.0) at test site 4, but only 8 (m.r. 0.5) of the animals were positive at test site 3. With acetone as vehicle, 7 animals (m.r. 0.4) were positive at test site 3 in the first challenge and 3 (m.r. 0.4) and 2 (m.r. 0.2) at test sites 1 and 6 respectively in the second challenge. With the mixture of petrolatum/acetone (90/10) as vehicle, 18 of 20 animals (m.r. 1.3) gave positive response at test site 4, while 6 of 20 guinea pigs (m.r. 0.4) were positive with alcohol as vehicle at test site 6.

II. CHEMICAL ANALYSIS

The retention time of the main peak of NPGDA in the HPLC-analysis was 6.2 min.

II:a. Adsorption. The average peakheight of the three injections of the solution containing filterpaper was 58.3 mm compared to 57 mm for the aluminium chamber solution and 58 mm for the NPGDA solution alone.

II:b. Polymerization. By making the peakheights a measure of concentration and by setting the concentration of the reference as initial, values counted as percentage of initial concentration of NPGDA could be estimated for the different solutions at different times. The values are summarized in Table 3. A diagram showing % of initial concentration of NPGDA versus time, for the acetone solutions, with and without inhibitor, is shown in Fig. 2.

II:c. Surface. The values for the filterpaper and glass surfaces compared with the aluminium surface are summarized in Table 4.

II:d. Petrolatum. The weight of the 0.2% w/w dispersion of NPGDA in petrolatum for the 2 h. extract was 0.0221 g. The amount of NPGDA is thus 4.42×10^{-5} g with a density of 0.828 kg/l of NPGDA (6), the corresponding volume of NPGDA is 5.34×10^{-5} ml. The dispersion in petrolatum was extracted with 1.0 ml acetone and 20 μ l of this extract was injected into the chromatograph and the resulting height of the main peak was found to be 18 mm. If no alteration of monomer takes place in petrolatum and no losses of monomer occurs as a result of the extraction procedure, a theoretical value of 1.07×10^{-6} ml of NPGDA in this 20 μ l fraction should be found. From comparison with the reference solution (20 μ l of 0.02% v/v NPGDA), which gave a peakheight of 108 mm for 4.0×10^{-6} ml of NPGDA, the petrolatum extract should give a theoretical peakheight of $\frac{108 \times 1.07 \times 10^{-6} \text{ mm}}{4 \times 10^{-6}}$

which is calculated to 29 mm. The peakheight found was 18 mm and this makes 62% of the theoretical value for the NPGDA amount. The same calculations were performed for the 24 h. petrolatum extract. The weight of the dispersion was 0.0254 g and the peakheight was found to be 15 mm. This makes 45% of the theoretical value of NPGDA.

DISCUSSION

There are many factors that can influence the elicitation of hypersensitivity in sensitized guinea pigs. The effect the vehicle could have on the test response, is probably the most important one. An inappropriate choice of a vehicle for the test substance will alter or even deplete the challenge reaction and thus give a false negative test response. A chemical reaction between the allergen and the vehicle is also a possibility as well as polymerization of the test substance. The penetration properties depend on the vehicle used. The evaporation of the vehicle or test substance might also effect the challenge response. Other main factors that might alter the test results are; possible adsorption of the allergen to the filterpaper or aluminium disc, skin metabolism of the allergen, interference of simultaneous skin test reactions and location of the test sites.

A great discrepancy between the test results in this study is seen when petrolatum and acetone are used as test vehicles. The number of positive animals as well as

the mean response figures are markedly decreased when the various acrylic compounds were tested in acetone (Table 1, 2). Quite a few of the acrylates would even be considered as non sensitizers if only acetone had been used as test vehicle. The intensity of the challenge response was greatly improved by using petrolatum instead of acetone, and the tests were definitely allergic and not irritant. This is supported by the fact that the test response in all control animals was negative (3, 4, 5).

The influence of the vehicle on the induction and elicitation of contact allergy has received little attention. Most of the investigators have reported on the effect the vehicle might have on the induction phase. Usually the investigator arbitrarily chooses, a solvent suitable for the actual purpose.

In the original description of the GPM-test (1, 2) the vehicle used for challenge was usually petrolatum, but water or propylene glycol were also used in particular cases. No explanation has been given to why those vehicles are more suitable than others.

2,4-Dinitrochlorobenzene (DNCB), the most commonly used experimental allergen, is usually dissolved in ethanol, acetone or olive oil for topical application. Often the same vehicle is used for both induction and elicitation. The vehicles used for sensitization with DNCB by injection have been ethanol, acetone or propylene glycol. Stevens (7) compared the effect of vehicles on sensitization to DNCB using the open technique for both induction and challenge. In declining order of effectiveness were dimethyl formamide, olive oil, paraffin oil, acetone, propylene glycol, glycerine and ethanol. In a limited study, Magnusson & Kligman (2) found that for topical sensitization to DNCB, p-nitroso-dimethylaniline (NDMA) and also to p-phenylenediamine (PPDA), 70% ethanol was superior to petrolatum, propylene glycol and acetone. For intradermal sensitization propylene glycol and peanut oil were equally effective with DNCB, but with NDMA and PPDA, peanut oil was superior. Propylene glycol was markedly more effective than 70% ethanol with all three allergens. In the case of PPDA, equivalent results were obtained with water and propylene glycol.

Chung & Giles (8) sensitized guinea pigs to methyl, and n-butyl methacrylates. Challenge with the methacrylates in olive oil gave strong positive reactions, but in alcohol it was impossible to elicit any allergic skin reactions at all. The authors believe that a high diffusion rate of the compound in the gaseous or liquid state

through tissue fluids or a high volatility rate into the air from the skin surface could be an explanation.

In several challenges, van der Walle et al (9), compared the effectiveness of petrolatum, arachis oil and Aramek (a mixture of 2 parts methyl ethyl ketone and 1 part of arachis oil by volume) as a vehicle for the mono(meth)acrylates they investigated. None of the vehicles was superior. Aramek, was preferred, as they found it easy to handle, the amount could be applied precisely and it had good adherence to the guinea pig skin.

Maurer (10) has discussed the influence of the vehicle on skin irritation of some chemicals in the optimization test in the guinea pig skin. The strongest irritation was found when the irritants were incorporated in petrolatum or in 2% carboxy-methyl cellulose.

Christensen et al (11) challenged DNCB sensitized guinea pigs with DNCB dissolved in alcohol, acetone and olive oil. Alcohol clearly gave the highest degree of positive reactions. Indicating that alcohol is more effective in eliciting a response than acetone.

In humans, Kligman (12) found that petrolatum is the most generally useful and effective vehicle in challenge patch testing both for water soluble agents as well as for lipid soluble agents. In the original Draize skin sensitization procedure for humans (13), no mention is made of the vehicle to be used. However, petrolatum is considered as vehicle of choice, when used in the modified Draize test (14, 15, 16).

In two tests in humans with costus oil, Marzulli & Maibach (17) proved alcohol to be more effective in eliciting a response than petrolatum, using the modified Draize test (16). On the other hand, in one test with cinnamic aldehyde, no difference in results was obtained with these two vehicles. The authors suggest the possibility that optimum test results might be achieved with a vehicle other than petrolatum. However, their investigations gave no evidence for the superiority of one vehicle in preference to another. Marzulli & Maibach, (18) found in further studies, that alcohol was shown to be a more effective vehicle than petrolatum, when fragrance ingredients were used in predictive tests in human subjects.

Ljunggren & Möller (19) found that chlorhexidine, which is soluble in aqueous but not in hydrocarbon solvents, gave a positive patch test reaction at a concentration

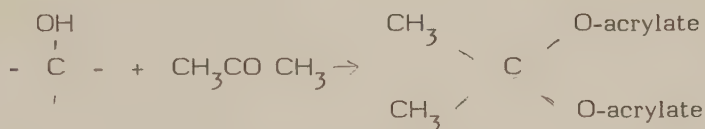
of 0.05% in water and 0.5% in alcohol, whereas results were negative at a concentration of 1% in petrolatum. However, petrolatum is the most suitable vehicle for human diagnostic patch testing, since it does not evaporate, protects against oxidation, and increases the shelf stability of the test substance (20, 21).

In this investigation we found a decrease in reactivity in animals sensitized to NPGDA when tested not only with acetone but alcohol as well, compared to petrolatum and the mixture of petrolatum and acetone (90/10) as vehicle (Table 2).

When changing the test site location we noticed a decrease in test response with the petrolatum vehicle from 15 to 8 positive animals and the mean response was decreased by half (Table 2). Thus, half of the animals did not react when the challenge test was applied near the abdomen (site 3) compared to a patch test site close to the back (site 1). No significant difference in the test response was observed with the acetone vehicle on the various test sites (Table 2). We have also noticed in previous investigations (3, 4, 5) that patch tests located more caudally on the flank of the animal (site 1, 2, 3) seem to elicit a slight increased reactivity compared to the ones more towards the head (site 4, 5, 6). Similar decrease in skin reactivity at the test sites towards abdomen compared to the ones towards the back has been found to apply to chlorcresol (22). These findings differ from those of Magnusson & Kligman (2) who compared the challenge response on the flank with those on the back and the abdomen. The back was compared with the scapula and sacrum. No significant differences were observed, except for reactions on scapula which were stronger than over sacrum.

Fig. 4 illustrates the concepts regarding chemical resistance involved in the vinyl ester resin structure adapted from Carey & Launikitis (23). The two points of vinyl reactivity, which are the cross-linking sites on cure (the acrylic or methacrylic moiety), and the two ester linkages are considered to be the point of greatest vulnerability to chemical attack. Ether linkages and methyl groups (shown as R) are considered to improve chemical resistance in themselves, and the placement of the highly stable methyl group serves to shield the ester group from chemical attack. Hydroxyl groups are known to contribute to good adhesion in polymer systems.

The effect of acetone on the dramatic quenching of the test reactions has no complete chemical explanation. According to the structure of the acrylates, none should react chemically with acetone (or any other ketones), except perhaps for those acrylic compounds containing hydroxyl groups in the backbone (the basic chemical structure without pendant groups). With these compounds acetone could give ketals (24), i.e.:



This could explain the drop in the eliciting response to some acrylates but not to others.

Organic solvents such as ethers, ketones and hydrocarbons, can easily form peroxides, and thus have influence on the polymerization of the acrylic compounds (25). Acrylates containing hydrocarbon chains $[(\text{CH}_2)_n \text{ recurring units}]$, will not form peroxides as easily as those containing ethylene ether groups $[(\text{CH}_2\text{CH}_2\text{O})_n]$, because of the lack of oxygen atoms in the backbone (26). Certain chemical bonds may undergo direct cleavage when the molecule is excited. This appears to be the case in, for example, the disulfide bond, the chlorine-chlorine bond, and the nitrogen-nitrogen linkage. The resultant fragments (free radicals) are highly reactive and may recombine or react with surrounding molecules.

Ketones have a weak absorption in the ultraviolet region (27). The energy absorbed by the carbonyl group can be transferred to the neighbouring carbon-carbon bond and cause cleavage to produce radicals which can initiate polymerization. With acetone, for example, some methyl radicals combine to give ethane and others abstract hydrogen from surrounding acetone molecules to give methane (28). The resulting acetone radical may thus initiate polymerization of the acrylic compounds. Acetone has been used as a photoinitiator for certain polymerization processes at room temperature (27).

Another important method for generating active free radical intermediates, is through hydrogen abstraction reactions. Ease of radical abstraction for a given substrate depends upon molecular structure. Alcohols, alkyl ethers and amines undergo very rapid hydrogen abstraction (29).

As seen from our results of the chemical investigations (section II:b, Table 3), there is a marked difference in the monomer residue with increasing time between the solutions with (darkness) and without (daylight) inhibitor. The preventive effect that the inhibitor has on the polymerization might explain this difference. The monomer content on the aluminium chamber when acetone is used as vehicle is approximately 8% of the initial amount after 1 h. The decrease in the challenge response and the monomer reduction discussed above, when acetone and alcohol are used as vehicles, could thus be explained by a possible polymerization process of the acrylic monomer, initiated through radical formation and/or hydrogen abstraction.

The influence the surface has on the monomer as a function of time, is seen in Table 4. The monomer decrease is more effected by the aluminium surface than the glass and filterpaper surfaces. Aluminium oxide always present on an aluminium surface, is a catalyst (30), and could therefore enhance a probable polymerization process.

When petrolatum is used as vehicle, the monomer content is reduced to 62% after 2 h. and after 24 h. 45% of the monomer remains. This slight difference between the 2 and 24 h. values, indicates that a rather high proportion of the monomer decrease might be due to losses in the extraction procedure. The availability of the acrylic monomer in the petrolatum vehicle at the test site after 24 h. application is only reduced by half and this could thus in part explain the high challenge response. The petrolatum vehicle probably prevents the monomer to polymerize. Smaller amounts of acetone dispersed in the petrolatum vehicle do not decrease the test response (Table 2).

The adsorption of the test substance to the filterpaper or to the aluminium chamber surface might also be an explanation to a decrease in the test response. However, our findings in section II:a, does not indicate that adsorption of the acrylic monomer to the aluminium surface or filterpaper disc occurs.

Several vehicles present obvious differences in penetration. Organic solvents such as acetone, increase the permeability of the horny layer by destroying the cellular proteins which constitute the cell membranes and internal keratinous fibers. However, alcohol does not affect the integrity of the horny layer. The effect solvents may have on penetration is controvercial (31).

Hydration increases the rate of diffusion of substances penetrating the skin.

Occlusion greatly enhances the penetration of the test material through the skin with all vehicles. Petrolatum, which is a complex mixture of hydrocarbons, may have a promoting effect on penetration in a manner similar to that of dimethylsulphoxide (DMSO) (31).

Both solvents and allergens may be volatile. Evaporation of the solvent results in an increase of the concentration of the test substances, which is undesirable both because of the risk of primary irritant reactions and of sensitizations as seen with use of high concentrations of some allergens. Evaporation of a volatile test substance may lead to false negative reactions. Petrolatum decreases the volatility, thereby resulting in greater skin exposure to the allergen. The more volatile species, are less likely to cause sensitization if they are in a vehicle which results in rapid evaporation. Therefore, the differences in the eliciting response in this investigation, might also be attributed to the volatility of the monomer in acetone vs. petrolatum. However, an exogenous solvent is not essential for penetration to occur. Solids dried on to the skin from a volatile solvent penetrate long after the exogenous solvent has evaporated.

The interference of two simultaneous skin test reactions of intermediate strength has been studied in the guinea pig by Blomberg et al (32). When two simultaneous delayed hypersensitivity reactions were elicited with different antigens, the risk of interference appeared to be rather small. When, however, the same antigen was used for both skin test, suppression of one skin test by the other was found. This suppression could not be reduced by introducing a larger distance between the two skin tests.

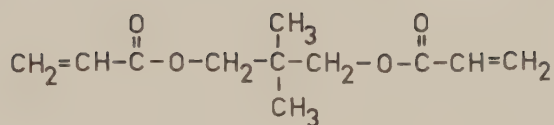
The influence of the test vehicle on the challenge response is not just a matter concerning the acrylic compounds. Fregert (33) compared in a GPM-test with Novalak the challenge reactions using petrolatum and acetone as vehicle. A significant decrease in positive tests was seen with Novalak in acetone.

Our investigation shows, that if only acetone had been used as a vehicle in the challenge procedure, some of the acrylic compounds tested, would apparently have been classified as non sensitizers. It must be emphasised that use of an inappropriate vehicle is one of the sources of low sensitivity and false negative reactions. The interaction between the vehicle, the allergen and the skin is obviously a complex phenomenon, which does not lend itself to simple generalizations. More knowledge and consideration is needed when to choose a suitable

vehicle for a test substance.

ACKNOWLEDGEMENTS

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NEOPENTYL GLYCOL DIACRYLATE (NPGDA)

Fig. 1. Chemical structure of neopentyl glycol diacrylate (NPGDA).

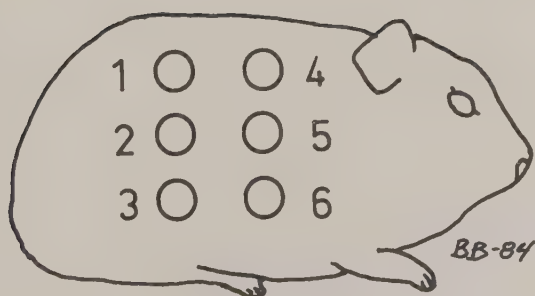


Fig. 2. Patch test location for challenge on the guinea pig flank.

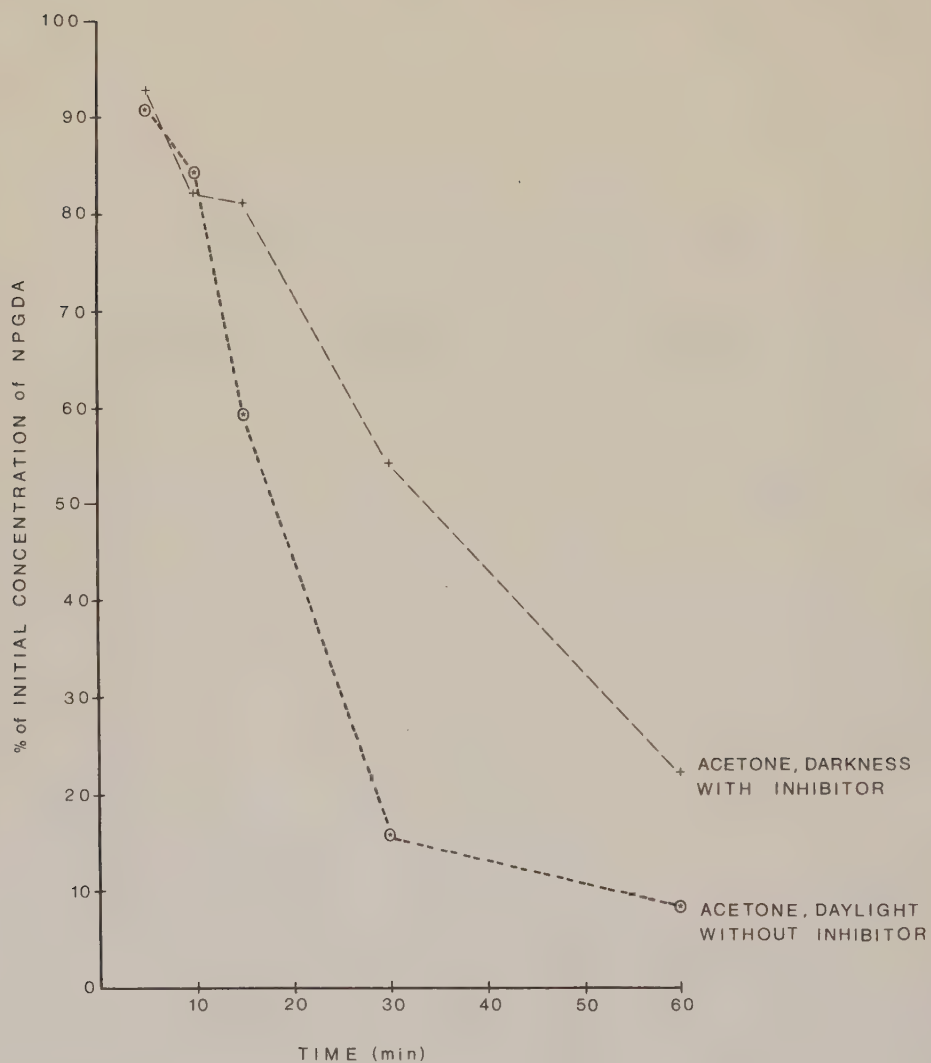


Fig. 3. The decrease of the NPGDA monomer on an aluminium chamber with time.

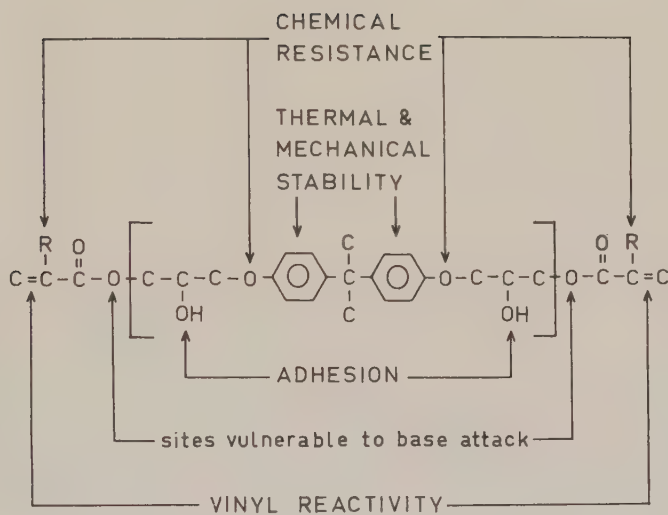


Fig. 4. Chemical resistance of a vinyl ester resin structure adapted from Carey & Launikitis (22).

Table 1. INFLUENCE OF THE VEHICLE ON THE TEST RESPONSE IN GUINEA PIGS SENSITIZED WITH VARIOUS ACRYLIC COMPOUNDS.

Acrylate used for induction		Number of positive animals of 15 tested	
		Petrolatum	Acetone
Prepolymers	Epoxy acrylate DOW	14	2
	Epoxy acrylate DRH-370	15	5
	BIS-PMA	0	0
	BIS-EMA	5	0
	BIS-GMA	13	0
	BIS-MA	10	4
	Ebecryl 220	2	0
	Ebecryl 270	13	4
	UEDMA	2	0
Multifunctional acrylates	PETA-3	10	0
	PETA-4	1	0
	OTA 480	6	0
	BUDA	10	7
	HDDA	9	1
	DEGDA	2	0
	TEGDA	0	0
	TPGDA	11	0
	NPGDA	14	2

Table 2. INFLUENCE OF THE VEHICLE AND PATCH TEST LOCATION ON THE TEST RESPONSE FOR NEOPENTYL GLYCOL DIACRYLATE (NPGDA)

a) = challenge 1.

b) = challenge 2.

Vehicle	Test site number	Positive of 20 tested	Mean response
Petrolatum	1 ^a	17	1.5
	4 ^b	15	1.0
	3 ^b	8	0.5
Acetone	3 ^a	7	0.4
	1 ^b	3	0.4
	6 ^b	2	0.2
Petrolatum/ acetone (90/10)	4 ^a	18	1.3
Alcohol	6 ^a	6	0.4

Table 3. INFLUENCE OF THE VEHICLE AND INHIBITOR ON THE ALTERATION OF MONOMER CONTENT ON AN ALUMINIUM CHAMBER AS A FUNCTION OF INCREASING TIME.

The numbers indicate % of initial concentration of NPGDA.

Vehicle & condition	Time (min.)				
	5	10	15	30	60
Acetone without inhibitor, daylight	91	84	60	16	8
Acetone with inhibitor, darkness	93	82	81	54	22
Acetonitrile without inhibitor, daylight	86	81	72	32	6
Acetonitrile with inhibitor, darkness	90	94	90	60	28

Table 4. INFLUENCE OF THE SURFACE ON THE ALTERATION OF MONOMER CONTENT AS A FUNCTION OF INCREASING TIME.

The numbers indicate % of initial concentration of NPGDA.

Surface	Time (min.)				
	5	10	15	30	60
Filterpaper	100	88	74	42	21
Glass	96	94	86	49	11
Aluminium	86	81	72	32	6

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